# GENETICS

A PERIODICAL RECORD OF INVESTIGATIONS
BEARING ON HEREDITY AND VARIATION

Founded in 1916
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Volume 35 JANUARY, 1950 Number 1

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PUBLISHED BIMONTHLY AT MENASHA, WISCONSIN,
BY THE BROOKLYN BOTANIC GARDEN, BROOKLYN, NEW YORK, U.S.A.
(Date of issue, January 10, 1950)

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Volume 35 JANUARY, 1950 Number 1

GENETICS is a bimonthly journal issued in annual volumes of about 600 pages each.

Subscription, $6.00 net a year for complete volumes (January-November). Foreign postage, 50 cents additional. Single copies, $1.25 each, postpaid.

As available, volumes 1 to 6 may be had at $9.00 each, and the later volumes at $7.50 each, postpaid. The Business Manager will supply information on request as to volumes and numbers available.

Correspondence concerning editorial matters should be addressed to the EDITOR OF GENETICS, Department of Zoology, University of California, Berkeley 4, Calif.

Business Correspondence, including change of address, directions concerning reprints, and exchanges, should be addressed to GENETICS, Menasha, Wisconsin, U.S.A., or BROOKLYN BOTANIC GARDEN, 1000 Washington Ave., Brooklyn 25, N.Y., U.S.A.

Remittances should be made payable to GENETICS.

Entered as second-class matter, August 31, 1922, at the postoffice at Menasha, Wisconsin, under Act of March 3, 1879. Acceptance for mailing at the special rate of postage provided for in the Act of February 28, 1925, paragraph 4, section 412, P.L. & R., authorized January 9, 1932.

Claims for missing numbers should be made within 30 days following their date of mailing. The publishers will supply missing numbers free only when they have been lost in the mails.

R. A. Emerson

# ROLLINS ADAMS EMERSON

## 1873–1947

ROLLINS ADAMS EMERSON, American Geneticist, son of CHARLES DAVID and MARY ADAMS EMERSON, was born at Pillar Point, New York, May 5, 1873 and died at Ithaca, New York on December 8, 1947. The results of his studies are published in some seventy odd papers—papers that are a permanent record of an active and productive scientific life.

At the age of seven EMERSON was taken by his parents to a prairie farm in Kearney County, Nebraska where he grew up and went to grade and high school. He subsequently attended the College of Agriculture of the UNIVERSITY OF NEBRASKA at Lincoln and there received his B.Sc. degree in 1897. After spending two years as Assistant Editor of Horticulture of the Office of Experiment Stations of the UNITED STATES DEPARTMENT OF AGRICULTURE in Washington, D. C., EMERSON returned to Lincoln, Nebraska as Assistant Professor of Horticulture in the College of Agriculture and as Assistant Horticulturist in the Experiment Station. He spent the academic year 1910–11 in graduate work at HARVARD UNIVERSITY with PROFESSOR EDWARD M. EAST. Harvard awarded him the D.Sc. degree in 1913. In 1914 he left his professorship at Nebraska and went to CORNELL UNIVERSITY as head of the Department of Plant Breeding of the College of Agriculture, a position he held until his retirement in 1942.

EMERSON was married in 1898 to HARRIET HARDIN, a sympathetic and understanding woman who helped greatly in creating a happy home in which four children, THERA, STERLING, EUGENE and MYRA were born and reared. Visits to the EMERSON home were not soon forgotten by the many students, associates and visitors who were privileged to make them.

EMERSON began his scientific career as a horticulturist but his interests soon shifted in the direction of genetics. He was one of America's outstanding pioneers in developing the new field in the years immediately following the rediscovery of MENDEL'S paper. His early work was on garden beans. In growing Indian corn for class material to illustrate Mendelian segregation he ran into a distorted ratio of starchy to sugary endosperm in a cross between white rice popcorn and a variety of sweet corn. His curiosity as to the basis of this unexpected result was undoubtedly influential in his replacing beans with corn as experimental material. In any event he and corn became inseparable.

The contributions of EMERSON to genetics were many and came at a time when the supports for the new science were weak, and the doubters many. His persistence and objectivity were great and he never published until he had extracted the truth from his experimental material and verified it not once but many times in many ways. Predecessors had studied the inheritance of plant and aleurone colors in corn and had been distracted by incidental modifying factors and apparent inconsistencies with Mendelian principles to the point

where some of them actually publicly renounced these principles. It was EMERSON'S persistence, clear thinking and hard-headed checking of facts which found the truth and established beyond any doubt that these apparently complex systems of inheritance have in reality a simple genetic basis. His papers on aleurone and plant color inheritance in maize are outstanding for sound experimental work, straight reasoning and clear presentation; they stand as inspiring models to all future generations of students. His investigations of the inheritance of quantitative characters, carried on partly in collaboration with PROFESSOR EAST, had an important influence on the thinking of geneticists. His studies on variegated pericarp led to the concept of unstable genes and represent another significant point in the history of genetics.

But important as were his direct scientific contributions, it is EMERSON the man who will be most vividly remembered by the many persons who knew him well. He was physically strong, well built and over six feet tall. He was cordial and warm in his relations with others. The contagious enthusiasm and zest so clearly displayed in his scientific work were extended to other activities. He hunted and bowled with vigor and evident enjoyment. During the corn season he prided himself on being the first to the garden and the last to leave, an example that without doubt increased the productiveness of all the students and research fellows who worked with him. It was the accepted practice to work from dawn to dark seven days a week during the pollinating season. Lunches in the garden house during these periods of intense activity were events of great influence on students. It was on such occasions that the unprinted lore of corn genetics was transmitted and EMERSON became best known to those who worked with him. It was through such informal contacts that EMERSON did his teaching. He gave no formal courses for many years, but he was nevertheless a really great and inspiring teacher—one who taught by example and friendly encouragement, not by lecturing. He was always available to students but he never directed their studies. They were treated as mature individuals and as equals.

EMERSON'S generosity is legendary. All his research materials were freely available to his own students and to other investigators the world over. Often he suggested thesis problems to students on which he himself had worked. But once such a problem was turned over to a student, it was then the student's responsibility. If a paper were published, it was the student's paper, not an EMERSON and DOE or even a DOE and EMERSON paper. This generosity played a very important part in making corn the best known of all plants from a genetic standpoint. It had the effect of interesting many people in many places in the material and in greatly speeding up progress. EMERSON derived as much pleasure from a good piece of research done by a student or a colleague as from one completed through his own efforts. This spirit of unselfishness combined with enthusiasm, fairness and a high order of ability naturally soon made him the intellectual and spiritual leader of the corn group—a group that grew and spread to include workers of many institutions and many countries who were not EMERSON students in the technical sense.

With the growth of the corn group the system of communicating unpublished information through conversation became inadequate. During 1932 at the Genetics Congress at Ithaca a "corn meeting" was held where it was decided that a central clearing house of information and seed stocks would be established at CORNELL. Out of this there evolved a series of mimeographed "corn news letters" edited by MARCUS RHOADES and sent to all interested corn geneticists. Later this became the "Maize Genetics Cooperation," a somewhat more formal organization for the dissemination of unpublished information and for maintaining seed stocks. At least to a certain extent the early corn news letters inspired the establishment by BRIDGES and DEMEREC of the analogous *Drosophila Information Service*, the first issue of which appeared in 1934.

During his life EMERSON received many honors. He was awarded the LL.D. degree by the University of Nebraska. He was a member of the National Academy of Sciences, the American Philosophical Society, and numerous other scientific societies. He served as President of the American Society of Naturalists in 1923 and as President of the Genetics Society of America in 1933. He was Dean of the Graduate School of CORNELL UNIVERSITY from 1925 to 1931. He was a member of the original editorial board of GENETICS at the time of its establishment in 1916 and served on the board until his death. Recognition and honors did not change EMERSON; he continued to the end to lead a simple, unpretentious life.

ROLLINS ADAMS EMERSON earned and had the admiration and respect of all those who came in contact with him. His own life was an effective, full and happy one and he enriched the lives of many others.

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# INHERITANCE IN NICOTIANA TABACUM: XXIII. DUPLICATE FACTORS FOR CHLOROPHYLL PRODUCTION

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Received April 7, 1949

WE HAVE previously reported (CLAUSEN and CAMERON 1944) studies of a chlorophyll deficient type, white-seedling, produced by GOODSPEED (1930) by X-ray treatment, which is inherited as a simple recessive in Purpurea, our standard variety of *Nicotiana tabacum*. In the 1946 planting season, however, it was observed that a varietal cross of Purpurea × Chinchao gave $F_2$ segregation in a ratio approximating 15 green:1 white-seedling. Fortunately this hybrid had been retained so that it was still available after segregation for white-seedlings had been observed in its offspring and we also had at the same time a standard Purpurea plant heterozygous for white-seedlings. The immediate progeny from intercrosses between the two, segregated for white-seedlings in a 7:1 ratio (table 2, Test cross), as was to be expected if the two types were identical genetically. These results establish a duplicate factor system for inheritance of green vs. white-seedlings.

## LOCATION OF THE FACTORS, $Ws_1$ AND $Ws_2$

In a previous report (CLAUSEN and CAMERON 1944) we have outlined the procedure employed in using monosomics for determining chromosome locations of factors. The application of this scheme to the two factors concerned in white-seedling inheritance may be outlined as follows. The monosomics are of the standard variety, Purpurea, which has the gametic formula, $ws_1Ws_2$, whereas Chinchao is $Ws_1ws_2$. Application of the monosomic method of determination first involves crossing the Purpurea monosomics individually with Chinchao, then testing progenies of the selfed $F_1$ monosomic types separately for white-seedling segregation. Let X and Y represent the two chromosomes concerned. Then $F_1$ haplo-X will have the constitution

$$\frac{X_0}{X - Ws_1}\,\frac{Y - Ws_2}{Y - ws_2},$$

and $F_1$ haplo-Y will be

$$\frac{X - ws_1}{X - Ws_1}\,\frac{Y_0}{Y - ws_2},$$

$X_0$ and $Y_0$ representing the missing X and Y chromosomes respectively. The normal hybrid selfed will segregate, 15 green:1 white-seedling, whereas $F_1$ haplo-X will not segregate at all for white-seedlings, since offspring must have at least one X-chromosome, and $F_1$ haplo-Y will segregate, 3 green:1 white-

TABLE 1

*White-seedling segregation in monosomic hybrids of Nicotiana tabacum var. Purpurea×var. Chinchao.*

| TYPE & GARDEN NO. | NO. OF SEEDLINGS | WHITE SEEDLINGS | PER CENT OF WHITE SEEDLINGS |
|---|---|---|---|
| haplo-A 48381p20 | 94 | 7 | 7.4 |
| B 48382p12 | 93 | 8 | 8.6 |
| C 48383p21 | 99 | 5 | 5.0 |
| D 48384p5 | 87 | 4 | 4.6 |
| E 48385p16 | 100 | 8 | 8.0 |
| F 48386p11 | 84 | 7 | 8.3 |
| diplo-G 48387p6 | 97 | 7 | 7.2 |
| haplo-G 48387p30 | 80 | 0 | 0.0 |
| | 86 | 0 | 0.0 |
| H 48388p16 | 95 | 8 | 8.4 |
| diplo-I 48389p1 | 92 | 4 | 4.3 |
| haplo-I 48389p26 | 75 | 0 | 0.0 |
| | 31 | 0 | 0.0 |
| haplo-I 48389p32 | 87 | 1 | 1.1 |
| | 87 | 4 | 4.6 |
| haplo-I 48389p40 | 85 | 6 | 7.1 |
| J 48390p43 | 85 | 6 | 7.1 |
| K 48391p3 | 98 | 4 | 4.1 |
| L | — | — | — |
| haplo-M 48393p6 | 91 | 3 | 3.3 |
| N 48394p14 | 97 | 5 | 5.2 |
| O 48395p16 | 96 | 8 | 8.3 |
| P 46372p4 | 99 | 10 | 10.1 |
| P 46372p10 | 97 | 3 | 3.1 |
| Q 48397p20 | 99 | 5 | 5.1 |
| R 48398p11 | 98 | 7 | 7.1 |
| S 48399p16 | 91 | 3 | 3.3 |
| diplo-T 48400p46 | 98 | 9 | 9.2 |
| haplo-T 48400p33 | 90 | 24 | 26.7 |
| U 48401p11 | 79 | 7 | 8.9 |
| V 48402p16 | 99 | 3 | 3.0 |
| W 48403p26 | 93 | 7 | 7.5 |
| Z 48404p5 | 93 | 7 | 7.5 |

seedling, since it is heterozygous for only one of the two loci. Obviously monosomics of chromosomes other than X and Y will segregate according to the duplicate factor scheme, 15 green:1 white-seedling and normal sibs of the monosomic types should segregate in the same ratio.

Results of segregation in the monosomic types are contained in table 1. The selected monosomic types were selfed and the seedling segregation ratios were determined by germinating 100 seeds in a water germinator, except in the case of resowing of 48389p26, for which only 45 seeds were available for the retest. All the monosomics except G and T segregate in satisfactory agreement with the 15:1 ratio. Of the two monosomics which behave exceptionally, haplo-G failed to segregate for white-seedlings and haplo-T gave a ratio of

3:1 instead of 15:1. The normal sibs in each case segregated in the 15:1 ratio, as was to be expected. The results conclusively demonstrate that the $Ws_1$ locus is located in the G chromosome, and $Ws_2$ in T. The latter result confirms our previous observation (CLAUSEN and CAMERON 1944), since, as a matter of fact, haplo-T had been isolated deliberately from an asynaptic type by using white-seedling as indicator of its presence in the cultures. These results unfortunately are somewhat marred by failure of the original haplo-I selection to segregate for white-seedlings. Two other haplo-I selections, however, were available, both of which segregated in the expected ratio. In conducting these experiments the original scoring of plants into monosomic and normal categories was based upon morphological features. However, each

TABLE 2

*Segregation for white seedlings in various lots of comparable composition.*

| LOT | | GREEN SEEDLINGS | WHITE SEEDLINGS | PER CENT WHITE SEEDLINGS |
|---|---|---|---|---|
| Varietal crosses | observed | 626 | 44 | 6.6 |
| | expected 15:1 | 628 | 42 | 6.25 |
| Monosomic tests | observed | 2090 | 136 | 6.1 |
| | expected 15:1 | 2087 | 139 | 6.25 |
| Diploid sibs | observed | 267 | 20 | 7.0 |
| | expected 15:1 | 269 | 18 | 6.25 |
| Test cross | observed | 87 | 14 | 13.9 |
| $Ws_1ws_1Ws_2ws_2 \times ws_1ws_1Ws_2ws_2$ | expected 7:1 | 88 | 13 | 12.5 |
| Haplo-T segregation | observed | 66 | 24 | 26.7 |
| | expected 3:1 | 68 | 22 | 25.0 |

such selection was later verified by cytological examination. It is usually not possible to await the results of this verification for seed collection, consequently the practice has been to make the necessary pollinations immediately and to collect seed from all selected plants. In the case of haplo-I, the cytological determinations indicate that haplo-I 48389p26 had the IM association, 17(2)+6+4+2(i); i.e., there were 17 bivalents, a ring of six, a ring of four and 2 univalents. This plant therefore was a double monosomic, apparently by rare chance, haplo-G haplo-I. Haplo-I 48389p40 was 18(2)+6+4+1, an unmodified haplo-I, whereas haplo-I 48389p32, although also 18(2)+6+4+1, had some undefined complication. The normal configuration of this hybrid is 19(2)+6+4, due to structural difference between the two parental varieties, Purpurea and Chinchao; but fortunately the chromosomes carrying $Ws_1$ and $Ws_2$ are not involved in these complications.

We have collected in table 2 the totals of segregating populations and have entered also the expected segregation values. In this table the monosomic lot

contains the totals for white-seedlings in all but haplo-G and haplo-T. The diploid sibs lot records the totals of segregation observed in normal sibs of haplo G, I, and T, individually recorded in table 1. The haplo-T segregation ratio is also included, and shown, like the rest, to be in very satisfactory agreement with expectation.

## THE CONSTITUTION OF *N. tabacum* VARIETIES

The demonstration that duplicate factors govern white-seedling segregation in the varieties Chinchao and Purpurea has aroused our curiosity as to the constitution of other varieties of *N. tabacum*. Obviously varieties may be of three categories, viz., duplex $Ws_1Ws_2$, simplex $Ws_1ws_2$ as in Chinchao, or simplex $ws_1Ws_2$ as in Purpurea. To determine the constitution of other varieties, parallel crosses were made of these varieties with Chinchao and Purpurea as testers; the $F_2$ results, as shown in table 3, are then diagnostic as to the constitution of the opposed varieties. The total segregation values for the crosses which produced white-seedlings are entered in table 2 as the varietal crosses lot. They are obviously in very satisfactory agreement with 15:1 expectations.

TABLE 3

*Diagnostic behavior of classes of varieties of N. tabacum, when crossed with the two tester varieties, Chinchao and Purpurea.*

| TESTER VARIETIES | CONSTITUTION OF UNKNOWN | | |
|---|---|---|---|
| | $Ws_1Ws_2$ | $Ws_1ws_2$ | $ws_1Ws_2$ |
| Purpurea $ws_1Ws_2$ | $F_1$ $Ws_1ws_1Ws_2Ws_2$<br>$F_2$ 1 green:0 white | $F_1$ $Ws_1ws_1Ws_2ws_2$<br>$F_2$ 15 green:1 white | $F_1$ $ws_1ws_1Ws_2Ws_2$<br>$F_2$ 1 green:0 white |
| Chinchao $Ws_1ws_2$ | $F_1$ $Ws_1Ws_1Ws_2ws_2$<br>$F_2$ 1 green:0 white | $F_1$ $Ws_1Ws_1ws_2ws_2$<br>$F_2$ 1 green:0 white | $F_1$ $Ws_1ws_1Ws_2ws_2$<br>$F_2$ 15 green:1 white |

As a result of these tests 25 varieties proved to be $Ws_1Ws_2$; 1 variety, Chinchao was $Ws_1ws_2$; and 8 varieties, inclusive of Purpurea, were found to be $ws_1Ws_2$, as shown in the following lists:

$Ws_1Ws_2$: Acomayo Red, Alameda Red, Ambalema, Apolo Pink, Apolo Red, Bergerac Catacorolla, Cachicadan, Canchaque, Cerro Alegre, Chulque Pink, Chulque White, Coquimbo, Coripata, Coroica, Cuba White, Huadquina, Huanuco, Marcapata, Maryland Mammoth, Mirador, Papago, Samsoun, Serrate, Trelease Turkish, Trujillo.

$Ws_1ws_2$: Chinchao

$ws_1Ws_2$: Belge, Ceniza, Consolation, Glutinosa-Resistant Kentucky Burley No. 1, GR Kentucky Burley No. 2, Holmes GR Samsoun, Purpurea, Station Standup Burley.

Thus most of the varieties in this relatively small sample, still possess the unmodified duplicate condition.

To what extent the eight varieties listed as $ws_1Ws_2$ are of independent origin

is unknown. However, three of them were burley varieties, presumably of common derivation. One of the other varieties was Holmes Glutinosa-Resistant Samsoun. Since the original Samsoun proved to be $Ws_1Ws_2$ and Holmes GR Samsoun was established by crossing Samsoun with an amphidiploid *glutinosa-tabacum* type derived from Purpurea, Holmes GR Samsoun must owe its $ws_1$ $Ws_2$ constitution to transfer of the $ws_1$ gene from Purpurea. We have no means of judging how the $ws_1Ws_2$ constitution may have been established in the other varieties of this class; whether by inheritance or by independent mutation; but probably the disparity in frequency of the two simplex types is not so great as that indicated by the raw data.

## DISCUSSION

If as seems well established, *N. tabacum* originated as an amphidiploid hybrid, then as a raw amphidiploid it must have contained an immense number of duplications, particularly of factors concerned with vital functions, as in this instance. In the amphidiploid, therefore, it should be possible for one member of each set of duplicate factors to mutate without impairing the operation of the system. In consequence, mutation pressure in the absence of counter selection might in time lead to loss of one or the other factor so that ultimately the species would become simplex as respects its duplicate factors; i.e., reduced to the constitution, $A_1a_2$, speaking in general terms. In such circumstances, FISHER (1935) has shown that under panmictic conditions, the dominant gene with the higher mutation rate would disappear and the population would become simplex for the remaining dominant.

In the autogamous species *N. tabacum*, however, this process should lead to establishment of two different simplex types approximately in proportion to their respective mutation rates. Thus if mutation rates $u_1$ and $u_2$ be assumed for factors $A_1$ and $A_2$, respectively, the relative gene frequencies after $n$ generations, in the entire absence of selection, would be as follows:

$$A_1 : a_1 = e^{-u_1 n} : 1 - e^{-u_1 n}$$

$$A_2 : a_2 = e^{-u_2 n} : 1 - e^{-u_2 n}.$$

If we ignore heterozygous conditions, which would be exceedingly rare under self fertilization, and assume that mutations at the two loci are independent and equal in rate, and allow for the elimination of double homozygous recessives, the approximate relative proportions of surviving genotypes would be:

$$\text{Duplex} \quad A_1A_2 = e^{-2un}$$

$$\text{Simplex} \quad A_1a_2 + a_1A_2 = 2e^{-un}(1 - e^{-un}).$$

Table 4 records the proportions of simplex lines to be expected in each set of duplicate factors, assuming that mutation frequency has been equal at each of the two loci. Conversely, these values may be taken to represent the relative proportions of sets of duplicate factors which have been reduced to the simplex condition under the stipulations indicated. We gratefully acknowledge the assistance of our colleague, DR. EVERETT R. DEMPSTER, in this portion of our studies.

The surprising feature of these results is the slowness with which reduction to the simplex condition will take place, as a consequence of mutation pressure alone. Mutation rates of the order of one in a million would only lead to establishment of simplex conditions in two percent of the lines after 10,000 generations. Since *N. tabacum* is an amphidiploid species, presumably established under cultivation, it is improbable that it has existed any longer than that. Moreover we have no reason to suspect that it has a mutation rate higher than

TABLE 4

*Proportion of simplex lines to be expected in the surviving population under stipulated assumptions as to mutation rate and number of generations.*

| MUTATION RATE | PROPORTION OF SIMPLEX LINES IN $n$ GENERATIONS | | |
|---|---|---|---|
| | $n=2000$ | $n=5000$ | $n=10{,}000$ |
| $u=10^{-4}$ | .307 | .565 | .775 |
| $u=10^{-5}$ | .039 | .093 | .174 |
| $u=10^{-6}$ | .004 | .010 | .020 |

that characteristic of other organisms. We have, however, some cogent evidence, derived particularly from monosomic studies, which indicates that numerous sheltered lethals, to use FISHER'S term, do exist in the species, probably in high enough frequency to be present in every chromosome. As a matter of fact, even if only one percent of originally duplicate loci scattered at random throughout the genetic system became reduced to the simplex condition, then possibly enough sheltered lethals would exist to account for the lethal effect of complete loss of any large segment of the germinal material.

Our experience with the F chromosome may be pertinent to this discussion. If we remove the non-satellited arm of the F chromosome by fragmentation, and replace it with material derived from another chromosome, the result is a viable type. If the other arm of the chromosome is removed, results seem to differ depending upon the size of the deletion. If it is large, removing most of the arm, a lethal effect is obtained; if, however, it is not quite so large, a non-lethal type is produced. Here the results seem to indicate the presence of a single sheltering factor lying close to the centromere in the satellited arm of the chromosome. Deletion of segments which do not contain this factor, do not lead to lethal effects, although they may lead to other, less-pronounced reductions in viability. Thus the process of diploidization, assumed to occur in amphidiploids, operates slowly and probably does not lead to actual diploidization to the extent that substantially all sets of duplicate genes are reduced to the simplex condition, but may proceed far enough even with a low mutation rate and a moderate number of generations to assure that each effective segment will contain a sheltering factor, which is all that is necessary to account for the genetic phenomena characteristic of established amphidiploids. This probably accounts for our failure to uncover other types of chlorophyll defect, in these crosses between normal green varieties. It also probably accounts for our ex-

perience that mutants sent to us as simple recessives often prove to depend upon a duplicate factor basis when tested with our varieties.

Presence of duplicate factors for chlorophyll production has recently been observed by MATSUMURA and KONDO (1942) in crosses between races of the tetraploid species, *Aegilops triuncialis*. PHILP (1935) in some earlier observations in effect demonstrated existence of duplicate loci for albinism in hexaploid oats *Avena saliva*. Recently AUSEMUS, HARRINGTON, REITZ, and WORZELLA (1946) have summarized observations of the occurrence of sets of duplicate factors in tetraploid and hexaploid wheat. None of these studies, however, reveals any information as to the frequency of the genes in question.

## SUMMARY

1. In *N. tabacum*, crosses between the normal varieties Chinchao and Purpurea segregate in a 15:1 ratio for green vs. white-seedlings in $F_2$, thus demonstrating existence of duplicate factors for chlorophyll production.

2. The white-seedling type for which this cross segregates is shown to be identical with or allelic to the white-seedling type established by GOODSPEED (1930) by X-ray treatment of Purpurea.

3. Monosomic tests demonstrate that one of the loci is located in the G and the other in the T chromosome.

4. A study of the constitution of a group of varieties revealed that 25 were $Ws_1Ws_2$, 1 $Ws_1ws_2$, and 8 $ws_1Ws_2$.

5. Attention is called to FISHER's (1935) theoretical treatment of the problem of sheltering of lethals in tetraploids, which leads to the conclusion that mutation pressure alone is relatively ineffective in reducing duplicate factor sets to a simplex condition. This conclusion appears to be substantially in agreement with conditions in amphidiploid *N. tabacum*, as far as present scanty evidence is concerned.

## LITERATURE CITED

AUSEMUS, E. R., J. B. HARRINGTON, L. P. REITZ, and W. W. WORZELLA, 1946 A summary of genetic studies in hexaploid and tetraploid wheats. J. Amer. Soc. Agron. **38**: 1082–1099.

CLAUSEN, R. E., and D. R. CAMERON, 1944 Inheritance in *Nicotiana tabacum*. XVIII. Monosomic analysis. Genetics **29**: 447–477.

FISHER, R. A., 1935 The sheltering of lethals. Amer. Nat. **69**: 446–455.

GOODSPEED, T. H., 1930 Inheritance in *Nicotiana tabacum*. IX. Mutations following treatment with X-rays and radium. Univ. Calif. Publ. Bot. **11**: 285–298.

MATSUMURA, S., and N. KONDO, 1942 Varietätsbastarde bei *Aegilops triuncialis* L. Bot. Mag. Tokyo **56**: 225–234. (Japanese with German résumé.)

PHILP, JAMES, 1935 Aberrant albinism in polyploid oats. J. Genet. **30**: 267–302.

# SPONTANEOUS BREAKAGE AND REUNION OF MEIOTIC CHROMOSOMES IN THE HYBRID BROMUS TRINII×B. MARITIMUS

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Received April 8, 1949

## INTRODUCTION

THE present investigation was begun when a preliminary survey of meiosis in the hybrid *Bromus Trinii×B. maritimus* showed not only numerous fragments, but a large number of chromosome bridges which could not be accounted for by the extent of chromosome pairing and the frequency of chiasmata. Bridges and fragments, not attributable to chiasma formation, have been found in mitotic and meiotic anaphase cells of various species following their exposure to irradiation and to certain chemicals; and such bridges and fragments have appeared spontaneously in mitotic anaphases of various forms. In these cases the bridges and fragments have been shown to result from breakage of the chromosomes with reunion of some of the broken ends in such ways as to produce dicentric chromatids.

Extensive chromosome fragmentation has been observed in meiosis in *Tulipa orphanidea* (DARLINGTON and UPCOTT 1941) and occasionally in Paeonia (J. L. WALTERS unpub.) and Lilium (EMSWELLER and BRIERLEY 1943). AKEMINE (1940), in a preliminary note, has described the occurrence in meiosis of Hosta of irregularities including fragments and bridges, which he attributes to chromosome rearrangements. HAGA (1937) reports bridges and fragments in *Kinugasa japonica* following transplantation from its subalpine habitat, and attributes these to fragmentation-fusion such as that induced by X-radiation.[1] However, there has so far been no detailed analysis of the spontaneous occurrence, in meiosis, of such numerous bridges and fragments which cannot be attributed to crossing over in heterozygous inversions. Therefore the present investigation was an attempt to determine the mechanism by which such bridges and fragments are produced in meiosis of the *B. Trinii×maritimus* hybrid, and whether this can be shown to correspond with the mechanism of bridge-formation following irradiation of chromosomes.

## MATERIALS AND METHODS

The cross *Bromus Trinii×maritimus* was made by DR. G. L. STEBBINS, JR., for the purpose of studying the phylogenetic relationships of *B. Trinii* and the *B. carinatus* complex. The results of this investigation are published elsewhere

[1] I found reference to a paper by T. HAGA, "Meiosis in Paris. II. Spontaneous break and fusion of chromosomes," (in press 1944). DR. HAGA has written me that this paper has not yet been published. Apparently he has observed in Paris spontaneous breakage and reunion similar to that in the Bromus hybrid which is the subject of the present study.

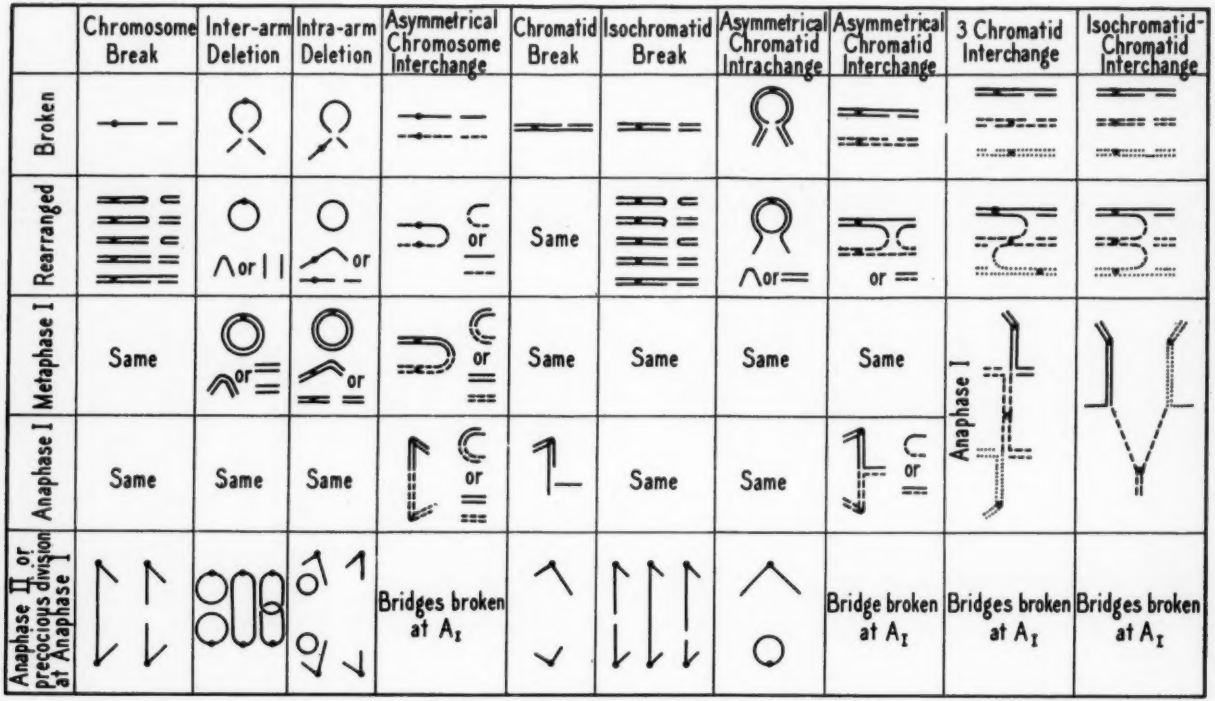


FIGURE 1.—Diagrams of various kinds of chromosome breakage and reunion occurring in meiosis. Adapted from LEA 1947, figures 30, 31.

(STEBBINS and WALTERS, in press). This paper contains descriptions of the morphology of the parent species and a more detailed description of the morphology of the hybrid than is given here.

The hybrids were grown in 1946; one collection was made in May from plant no. 684–1, grown in the greenhouse, and one collection in June from plant no. 684–8, grown in the field. All the completely analyzed metaphase cells except two and all the remaining data on meiosis were obtained from plant 684–8.

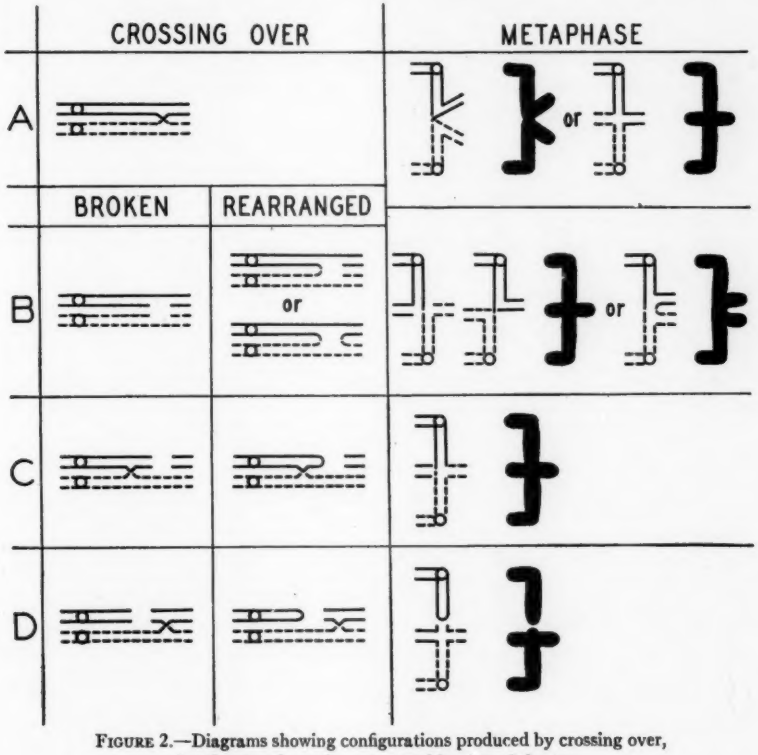


FIGURE 2.—Diagrams showing configurations produced by crossing over, breakage and reunion, or a combination of the two.

Both plants appeared to have the same type of metaphase pairing and both had bridges and fragments, but almost all the material from plant 684–1 was quite sticky.

The allopolyploids, obtained from colchicine treatment of hybrid plants (STEBBINS 1949) were grown in the field in 1947 and 1948. The allopolyploid plants ($C_3$) used in this study were the second generation from selfed seed, collected from the 12 most fertile plants of the preceding generation ($C_2$).

The fixations for cytological study were made in a mixture of 3 parts abso-

lute alcohol:1 part glacial acetic acid, and the material was stored in 70 percent alcohol. Meiosis was studied in pollen mother cell smears made with iron-acetocarmine.

## MEIOSIS IN THE PARENTS

The author is indebted to DR. G. L. STEBBINS, JR. for the following unpublished descriptions of meiosis in the parent species and of the morphology of the interspecific hybrid.

*B. Trinii* (2n=42, KNOWLES 1944) exhibits very regular meiosis, with 21 pairs of medium sized, morphologically indistinguishable chromosomes. There

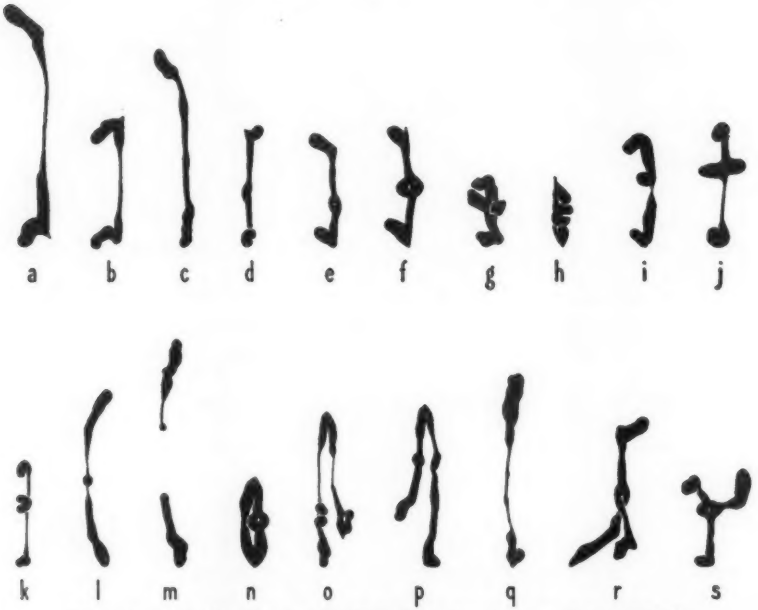


FIGURE 3.—Metaphase bivalents and trivalents observed in the hybrid. ×1300.

are generally two to three chiasmata per bivalent, one or two in each arm. This species has approximately 95 percent of stainable pollen.

*B. maritimus* (2n=56) also exhibits completely regular meiotic divisions, with 28 bivalents always present. Seven of these bivalents are much larger than the remaining 21 pairs; none of the chromosomes within these two groups can be distinguished from each other morphologically. The chiasma frequency is approximately two per bivalent, generally with one chiasma in each arm. This species has approximately 90 percent of stainable pollen and is highly fertile.

## MORPHOLOGY OF THE HYBRID

The hybrid (2n=49) was a sturdy plant, with a well developed root system;

it was more vigorous than the parent species. The hybrid population of 20 plants was strikingly homogeneous, with no evident morphological differences. No stainable pollen was observed, and these hybrids were completely sterile.

## MEIOSIS IN THE HYBRID

### *Prophase*

The cell shown in figure 4 represents the closest approach to a normal pachytene stage which was found. It may be observed in this cell that the majority of the chromosome segments appear to be single threads, which are believed to represent unpaired chromosome segments; several regions which are thicker and which appear to be double are interpreted as paired chromosome segments. There is some variation in the amount of pairing found at pachytene; however, the cell shown in figure 4 exhibits an average amount of pairing. It may therefore be concluded that very little chromosome pairing takes place in this hybrid.

There is no visible evidence of fragmentation at pachytene, either in the form of free fragments or as breaks in the chromosomes.

No diplotene or diakinesis cells were found, with the exception of a few "sticky" cells. Two slides were made on which an unusual distribution of stages was found, *i.e.*, from pachytene to metaphase. In almost all these cells, the chromosomes appeared to be sticky or clumped or both. Free fragments were observed in some prophase cells on these slides, but not in the metaphase cells.

### *Metaphase*

The seven large chromosomes of *B. maritimus* may be identified at metaphase in the hybrid, but the remaining 42 chromosomes do not differ enough in size to be distinguishable from one another. Configurations observed were univalents, bivalents, and trivalents (fig. 15); the different types of bivalents and trivalents which were found are shown in figure 3. Bivalents and trivalents are generally oriented at the equator of the cell, whereas univalents are scattered throughout the cell. Many of the univalents are bent, apparently at the centromere, so that they appear to be U-shaped.

Metaphase cells were difficult to analyze completely because of the large number of chromosomes and the small amount of pairing. The data on pairing and chiasmata presented below were compiled from 13 cells which could be completely analyzed. These data are supported by observations on 25 additional cells, which could not be completely analyzed but from which an estimate of the amount of association could be made.

The number of bivalents and trivalents per cell ranges from 0 to 5, with an average of about 4 per cell (it is possible that as many as 7 or 8 bivalents and trivalents may have been present in some of the unanalyzed cells). Trivalents account for a very small proportion of this figure, since only five were found in the 13 cells. The chiasma frequency is low, 0.56 per chromosome in bivalents and trivalents, and the terminalization coefficient is 0.50. The majority of the bivalents had only one chiasma, and all the trivalents had two chiasmata.

Only three of the 45 bivalents found in the 13 cells had two chiasmata; in each case there was one chiasma per arm. No bivalent or trivalent was observed with two chiasmata in one arm. It will be shown below that some of these bivalents and trivalents are probably the result of chromosome breakage and reunion rather than of chiasma formation; therefore the chiasma frequency is probably even lower than the figure given here.

Very few free fragments are observed at metaphase. The few that have been found appeared to have the same circumference as the chromosomes. However, chromosome constrictions were frequently observed (fig. 15, B, C).

### *Anaphase*

The number of fragments increases very slightly at the beginning of anaphase, when the two chromatids of each chromosome are distinct but still lie close together. However, as soon as the chromatids begin to separate, the number of fragments increases markedly, and by the time the sister chromatids have attained their maximum separation, each cell contains numerous fragments (fig. 18).

The following types of fragments were found: (1) *single fragment*: the very small single fragment is spherical, and larger fragments are rod-shaped. The single fragment may lie free in the cytoplasm or it may be attached to a chromosome arm; a complete gradation has been observed from a constriction in a chromosome arm to a fragment attached by a chromatin thread to the end of a chromosome arm (fig. 18). (2) *U-shaped fragment*: this term is applied to a rod-shaped fragment bent into a U form; however, it is possible that a minute U-shaped fragment may appear to be spherical. (3) *Paired fragments*: these are either closely associated free fragments of equal size (fig. 18E) or two fragments of equal size attached to the same end of a chromosome, each fragment being attached by a strand to the end of one chromatid arm.

Because of the numerous small chromosomes and frequent precocious division of univalents (fig. 18 D), the material was not suitable for precise analysis of the sizes, frequencies, and types of fragments. Fragments were observed to be attached to both short and long arms of the chromosomes, and one univalent had two terminal fragments, one attached to each arm of the same chromatid. In 28 early- and mid-anaphase cells the maximum number of fragments in any cell was 28, 10 free and 18 attached fragments (constrictions are not in-

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FIGURE 4.—Hybrid, pachytene cell showing very little chromosome pairing; arrow points to paired region. ×1300.

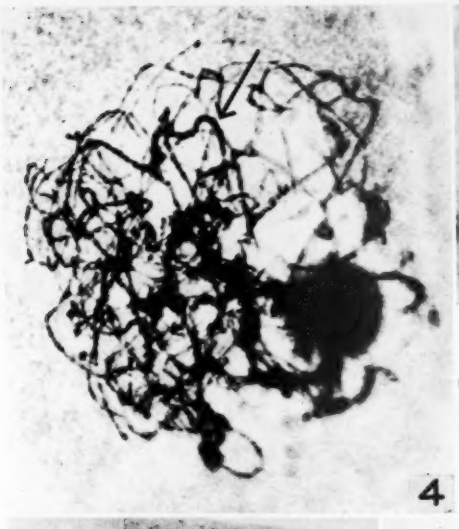
FIGURE 5.—Hybrid, first anaphase cell; arrow points to dicentric univalent chromosome resulting from precocious division and movement to poles of the centromere of a univalent loop chromosome such as that shown in Figure 18, C. ×1300.

FIGURE 6.—Hybrid, one cell of second anaphase-telophase dyad, showing a dicentric loop chromosome. ×1300.

FIGURES 7A, B.—Hybrid, first telophase cell showing five univalent bridges and a sixth structure in the form of a large loop. ×1300.

FIGURE 8.—Hybrid, second telophase dyad showing eight bridges. ×1300.

FIGURE 9.—Allopolyploid, metaphase cell of 98-chromosome plant, showing $46_{II}+4_{I}$. ×600.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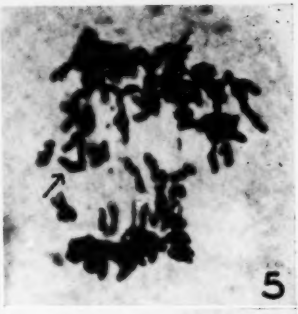
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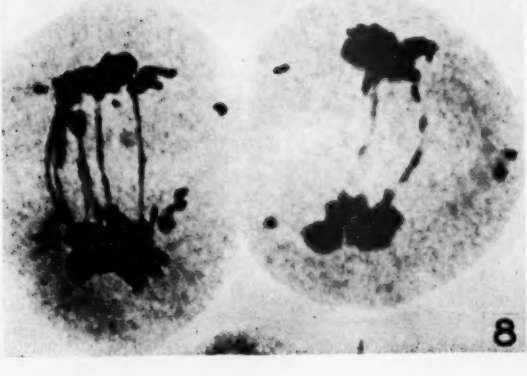
7A

7B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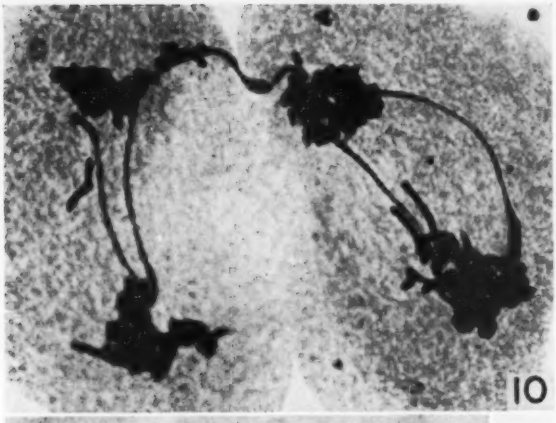
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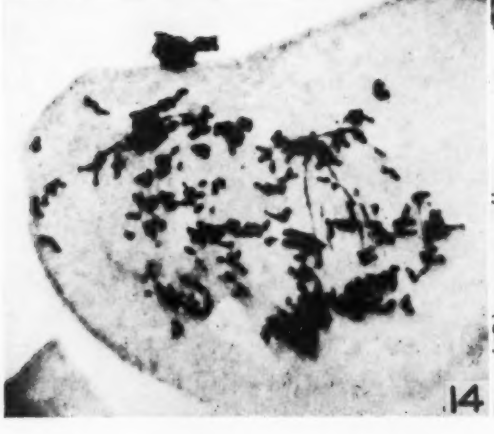
11A

11B

12

13A

13B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cluded in the counts because of the difficulty of interpreting borderline cases). The largest number of free fragments observed in a first anaphase cell was 15. These are minimum frequencies, since many fragments must have been covered by chromosomes and since uncertain fragments were not counted. Although no exact comparison of the frequencies of the different types of fragments described above can be made, it can be stated that attached and free single fragments are much more numerous than attached and free paired fragments. Judging from the relatively small size of the free and attached fragments, many of the breaks were in the distal regions of the chromosomes, some extremely close to the ends of the arms.

Frequency and types of bridges were studied in 50 first anaphase cells. Only early- and mid-anaphase cells were used, in order to avoid the possibility of loss of bridges by breakage or non-disjunctional movement to the poles, and to observe as far as possible the effect of precocious division of first anaphase chromosomes. Two types of bridges were found: The "bivalent bridge" (2–2), in which the chromosome at each end of the bridge consists of two chromatids, one arm of one of these included in the bridge (fig. 16); and the "univalent bridge" (1–1), which has only one chromatid at each end, with one of its arms involved in the bridge (fig. 18). An unusual configuration, which was observed twice, is shown in figure 17: three chromosomes are attached by means of two bivalent bridges. No "double bivalent bridges" were found; that is, no pair of chromosomes connected by two bridges.

The majority of the first anaphase cells examined contained one or more bridges. The average number of bridges per cell was approximately 1.1; the range was 0–4 (fig. 16). It was possible to identify 22 of the 47 bridges as bivalent or univalent bridges; 16 or 73 percent of these bridges were bivalent bridges (2–2), 5 or 23 percent were univalent bridges (1–1), one appeared to be bivalent at one end and univalent at the other (2–1) (fig. 18). Ten of the remaining bridges were oriented so that only one end could be observed; of these 8 were 2-? and 2 were 1-?. Thus, it may be estimated that 70–80 percent of the bridges are bivalent bridges, and 20–30 percent are univalent bridges. The 2–1 bridge is interpreted as the result of precocious division of the chromosome at one end of a bivalent bridge. The precocious division of lagging univalent chromosomes at first anaphase is a common occurrence in grass hybrids (KIHARA 1931), and evidently this precocity extends also to separated members of bivalents. The question arises as to how many of the so-called univalent bridges may have been produced by such precocious division of the chromo-

FIGURE 10.—Hybrid, second telophase dyad showing four second division bridges, and, at the top, a first division bridge. ×1300.

FIGURES 11A, B.—Hybrid, one cell of a dyad in second telophase, showing a loop chromosome with both arms visible in the nucleus, and three bridges. ×1300.

FIGURE 12.—Allopolyploid, early first anaphase cell of a 98-chromosome plant, showing complete regularity and lack of fragmentation. ×600.

FIGURES, 13A, B.—Hybrid, first telophase cell showing small loop chromosome configuration and univalent bridge. ×1300.

FIGURE 14.—Allopolyploid, early first anaphase cell of 96-chromosome plant, showing bridges, numerous fragments, and precociously divided chromosomes. ×800.

some at each end of a bivalent bridge. Since 2–1 bridges might be expected to be twice as frequent as such false univalent bridges, and only one of 22 bridges was a 2–1 bridge, it seems likely that at this stage the majority of such bridges as appear to be so are actually univalent bridges.

At late anaphase there is a decrease in the number of visible fragments; those which are attached to chromosomes are probably carried to the poles. The bridge frequency is very slightly lower; 28 bridges were observed in 30 cells, or approximately 0.9 bridges per cell. Of these, 12, or 40 percent, can be identified as univalent bridges; since many are uncertain, the percentage may be higher than this.

A late anaphase cell with six univalent bridges is shown in figure 7A, B. One of these bridges is in the form of a loop; this loop will be described below under Second Division, since the majority of such configurations were found at second telophase.

### *Telophase*

First telophase cells exhibited lagging chromosomes, fragments, and micronuclei; there were very many more fragments than lagging chromosomes. In the 25 cells examined, there were 22 bridges; 10 of these were broken.

### *Second Division*

At early- to mid-anaphase the fragment number was decreased because in many cases fragments were contained in the form of micronuclei in one or more small supernumerary cells.

Late anaphase and early telophase dyads were studied with reference to bridge frequency. An extremely conservative count of these bridges was made; any structure that was at all doubtful was excluded from the count. The figures reported here therefore represent the minimum number of bridges in late anaphase and early telophase dyads. Not a single dyad was found which did not contain at least one bridge. The average number of bridges per dyad was 3.5; the range was from one to eight bridges per dyad (figs. 8, 10).

Approximately 10 percent of the second anaphase-telophase cells contained a chromosome in the form of a loop; in all cases the short arms of this chromosome were included in the nucleus, but judging from the appearance of the loop portion of the chromosome the centromeres must lie close together, and in figure 11A, B, where the short arms can be distinguished in the nucleus, it may be observed that they do lie close together. Two late first anaphase cells were observed to contain loop chromosomes which, it is believed, are the same type as those described above (figs. 7A, B; 13A, B). In both cases the two centromeres are separate but lie close together (especially in figs. 7A, B); each loop appears to be of approximately equal thickness throughout. There is a pronounced difference in size of these loops; it may be that the large loop is formed from one of the seven large *B. maritimus* chromosomes.

It is not surprising that the sporads of this hybrid exhibit a great deal of irregularity. Only a few had four cells; the range was from 4 to 11 cells, the average 5.9 cells per sporad. The number of micronuclei per sporad ranged

from 4 to 19, with an average of 11.5 per sporad. In 7 of the 50 sporads observed, there was either a bridge or the remains of a bridge. Rarely there was a pair of very small, solidly stained round chromatic bodies lying very close together; it may be that these are the paired fragments first observed at first anaphase.

### MEIOSIS IN THE ALLOPOLYPLOIDS

The *B. maritimus-Trinii* allopolyploids are described fully in another paper (STEBBINS and WALTERS, in press); only those data pertinent to the condition of abnormal meiosis in the hybrid are given here.

Fluctuation of the chromosome number has taken place in these allopolyploids in the two generations since their production. The six plants investigated had the following numbers: 96, 98, 99 (3), and 100. From the type of metaphase configurations found in the 98-chromosome plant it could be concluded that this complement was deficient in one chromosome and duplicated in another.

Metaphase was very regular in all these allopolyploids. In the 96-chromosome plant the most complex configuration found was a quadrivalent, and the maximum number of multivalents observed in any cell was two quadrivalents. The maximum number of univalents per metaphase cell was five (including those observed in otherwise unanalyzed cells). Twelve of the 18 cells analyzed contained no univalents. In the 98-chromosome plant the most complex configuration found was a quadrivalent, and the maximum number of multivalents observed in any cell was two, $1_{IV}+1_{III}$; the majority of the cells contained one trivalent. The maximum number of univalents per cell was 10. Of the 50 cells analyzed, 78 percent contained 1–3 univalents, 22 percent contained 4–10; because of the duplication of one chromosome and deficiency of another, no metaphase cell contained $49_{II}$. No fragments were observed at metaphase.

Because of the large chromosome numbers, exact determinations of chiasma frequencies could not be made. However, the chiasma frequency is approximately two per bivalent. There is usually one chiasma in each arm, but some bivalents have only one chiasma and some have three, with two in one arm. A typical metaphase cell is shown in figure 9.

With rare exceptions the first anaphase cells appear to be perfectly normal; a typical first anaphase cell is shown in figure 12. Out of 100 early anaphase cells studied, a single cell was found in the 96-chromosome plant which exhibited as much fragmentation, bridge-formation, and precocious division of univalents as any anaphase cell observed in the hybrid (fig. 14); this was the only extremely irregular cell found in any of the allopolyploids, although 100 early anaphase cells of the 98-chromosome plant were studied as well as 50 cells of the 100-chromosome plant and 20 cells of each of two 99-chromosome plants.

Free single fragments and single attached fragments were observed in occasional anaphase cells; one pair of fragments was found in the 100-chromosome plant, and a U-shaped fragment in a cell containing a bridge. The large number of chromosomes makes exact determinations impossible; however, a reasonably accurate estimate of the number of fragments can be made. In the 96-chromo-

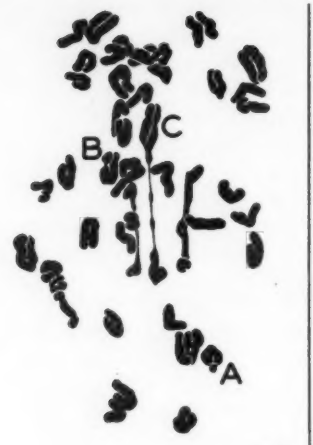


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FIGURE 15.—Hybrid, metaphase cell showing one trivalent, two bivalents, and 42 univalents; A=free fragment, B=constriction in a univalent chromosome, C=constriction in a trivalent configuration. ×1300.

FIGURE 16.—Hybrid, early anaphase cell, showing four bivalent bridges, and fragments. ×1300.

FIGURE 17.—Hybrid, early anaphase cell, showing triangular configuration composed of three chromosomes connected by two bridges, and attached and free fragments. ×1300.

FIGURE 18.—Hybrid, mid-anaphase cell showing bridges and numerous fragments; A=univalent bridge; B=univalent-bivalent bridge; C=univalent loop chromosome resulting from isochromatid or chromosome breakage followed by reunion of sister chromatids of centric segments; D=precociously divided univalent chromosome; E=paired fragments; F=attached fragment; G=stretched centromere. ×1300.

some allopolyploid, approximately 3 percent of the early anaphase cells contained 1–3 free fragments, and 3 percent contained 1–2 attached fragments; altogether, about 4 percent of the early anaphase cells contained attached or free fragments or both. In the 98-chromosome allopolyploid, one cell was found with one bridge and two free fragments, as well as three attached fragments; approximately 3 percent of the early anaphase cells contained 1–3 attached fragments.

In the 98-chromosome plant 5.5 percent of the first anaphase cells (100 early- plus 100 late-anaphase cells) contained a bridge, and in the 96-chromosome plant, outside of the one exceptional cell in which several bridges were observed, 5 per cent of the first anaphase cells contained one bridge and 0.5 percent contained two bridges. No univalent bridges were found. No bridges were found in 43 mid-late second anaphase cells in the 98-chromosome plant. Unfortunately only 17 late second anaphase cells were found in the material obtained from the 96-chromosome plant, but three, or about 18 percent, contained one bridge.

## INTERPRETATION OF DATA

At metaphase in the hybrid there are approximately 4 bivalents and trivalents per cell, with two chiasmata per trivalent and generally one chiasma per bivalent; at first anaphase there is approximately one bridge per cell. Since a single cross-over in a heterozygous inversion can produce a first anaphase bridge, the pairing and chiasma frequency at metaphase is sufficient to account for the number of first anaphase bridges observed, assuming a high degree of inversion heterozygosity in the hybrid. However, approximately 20–30 percent of the first anaphase bridges are univalent bridges, and since it has been shown above that these cannot be attributed to precocious division of bridge chromosomes, their presence cannot be explained on the basis of crossing-over in heterozygous inversion regions. Furthermore, each first anaphase cell, whether or not it contains a bridge, exhibits fragments; the number of fragments at first anaphase and at successive stages is always much larger than the number of bridges.

At second anaphase there is an average of 3.5 bridges per dyad and a range of 1–8 bridges per dyad. This high frequency of second division bridges cannot be explained on the basis of pairing and crossing over in heterozygous inversions. A cross-over proximal to a paired heterozygous inversion region, which is disparate in relation to a cross-over in the inversion region, produces a loop chromatid at first anaphase and from this a bridge at second anaphase; a cross-over proximal to the inversion, which is disparate in relation to complementary chiasmata in the inversion, produces two loop chromatids at first anaphase and thus two bridges at second anaphase (RICHARDSON 1936). However, the production of a large number of second division bridges by such means requires extensive metaphase pairing and a high chiasma frequency, since a minimum of two chiasmata per chromosome arm is required to produce a loop at first division and a bridge at second division. And it has been observed in this hybrid not only that there is very little chromosome pairing at metaphase and that the chiasma frequency is very low, but that in no case were

two chiasmata found in one arm of a bivalent or trivalent. This situation cannot be explained by complete terminalization of a large number of chiasmata, since very little pairing is observed at pachytene.

It may be suggested that some of the second division bridges result from nondisjunction of two bridge chromosomes at first anaphase; however, such nondisjunction could account for only a very small proportion of the second division bridges observed, since even at early first anaphase very few bridges are so located that both bridge chromosomes might be included in one nucleus.

Bridges and fragments, not attributable to chiasma formation in heterozygous inversions, have been reported in many materials following irradiation or chemical treatment. Such bridges and fragments have been observed in mitotic anaphase cells following exposure of earlier stages of mitosis to irradiation in Tradescantia (SAX 1938, 1940; SAX and MATHER 1939; LEA and CATCHESIDE 1942; and others), in Chortophaga (CARLSON 1938, 1941) and Chorthippus (CREIGHTON 1941); and in meiotic anaphase cells following irradiation of earlier stages in Nicotiana (GOODSPEED 1929), Tulipa (STONE 1933), Tradescantia (RILEY 1936, SAX 1938), Gasteria (MARSHAK 1935), Vicia (MARSHAK 1939; MARSHAK and MALLOCH 1942), and in Habrobracon (WHITING 1945a, b). Chemical treatment has been effective in producing bridges and fragments in mitotic and meiotic anaphases of Tradescantia (DARLINGTON and KOLLER 1947).

Bridges and fragments, apparently identical with those produced by irradiation, have also appeared spontaneously in mitotic anaphases, namely in root tips of Triticum×Secale hybrids (PLOTNIKOWA 1932); in the first post-meiotic mitosis of several Tradescantia species and in the $F_1$ and $F_2$ of a hybrid between *T. canaliculata* and *T. humilis* (GILES 1940); in the first post-meiotic mitosis of diploid and tetraploid Tradescantia species and their triploid hybrids, Tulipa, and diploid and triploid Hyacinthus (DARLINGTON and UPCOTT 1941).

In all the cases of bridge-formation cited above, it has been demonstrated that the bridges and fragments result from breakage of the chromosomes, followed by reunion of some of the broken ends in various ways to produce dicentric chromatids. SAX (1938, 1940), CATCHESIDE (1945), CATCHESIDE, LEA, and THODAY (1946), LEA (1947), and others have described the manner of production of such structural changes in chromosomes following their irradiation, and this will be summarized briefly here.

Irradiation has been shown to produce two types of chromosome alterations: (a) physiological, the change in the surface of the chromosomes which causes stickiness, and (b) structural, the breakage of chromosomes which may be followed by their reunion in various ways.

There are two types of structural change, the *chromosome break*, a break produced at the time when the chromosome is effectively single, and the *chromatid break*, produced when the chromosome is effectively double, and involving one or both sister chromatids (see fig. 1).

Following a chromosome or chromatid break, the majority of the newly broken ends undergo restitution to the original chromosome structure, while

a smaller proportion joins with other broken ends in the cell to form various types of structural rearrangements. A very small proportion of the original chromatid and chromosome breaks remain as such at metaphase.

Chromatid breaks may be single, or the two sister chromatids may be broken at the same locus; the latter is an *isochromatid break* (LEA and CATCHESIDE 1942). If it does not undergo restitution or take part in a chromatid exchange, the single chromatid break may result at anaphase in a terminally deleted chromosome and a fragment. Those isochromatid breaks which do not undergo restitution of one or both chromatids, or participate in a more complex exchange, have the following possibilities (see fig. 1): (a) sister-reunion of the broken ends of the centric segment, producing a dicentric chromosome and thus a bridge at anaphase, or non-union of these ends, producing two deleted chromosomes at anaphase; (b) sister-reunion of the broken ends of the acentric segment, producing a U-shaped fragment, or non-union, producing paired fragments.

It may be noted here that DARLINGTON and KOLLER (1947) maintain that isochromatid breaks are "fictitious," that such breaks result from division or reproduction of a chromosome which was broken when single, and that only chromatid breakage and reunion occur after the chromosome is double. However, evidence for the occurrence of isochromatid breaks is described by CATCHESIDE (1945).

In Tradescantia the sister chromatids formed by division of a broken chromosome and fragment do not fuse at the breakage points, so that no bridge is formed but instead two deleted chromosomes and two fragments (SAX and MATHER 1939; SAX 1940). However, in Chortophaga (CARLSON 1941) such chromatids and fragments formed by division of a broken chromosome and fragment have the same potentialities as listed above for isochromatid breaks: if there is fusion of sister chromatids of a centric segment at the breakage point a dicentric chromosome is produced which will form a bridge at anaphase.

Chromosome and chromatid interchanges may also produce anaphase bridges and fragments. A single chromosome break in each of two separate chromosomes produces four ends which may recombine so as to give rise to a dicentric chromosome and one or two acentric fragments. Such an interchange, known as an *asymmetrical chromosome interchange*, would result in the formation of a double bridge at anaphase and two U-shaped or paired fragments (fig. 1). If a chromatid break occurs in each of two separate chromosomes, and the two centric segments unite, a dicentric chromosome is produced which will form an anaphase bridge; this bridge is accompanied by a U-shaped fragment if the two acentric segments unite, or if not, by two single fragments. Such a rearrangement is known as an *asymmetrical chromatid interchange* (fig. 1).

Various other types of chromosome alterations have been described and illustrated by SAX (1940), CATCHESIDE (1945, 1948), CATCHESIDE, LEA, and THODAY (1946); LEA (1947) and others. Those alterations in chromosome structure which could have been detected in meiosis of the *B. Trinii*×*maritimus* hybrid are shown in figure 1.

The structural changes described above occur in both mitotic and meiotic

chromosomes. In this respect a mitotic chromosome, which exhibits a bridge at anaphase, corresponds to a meiotic univalent chromosome, in which the bridge will be apparent at first anaphase if the chromosome lags and divides precociously, or at second anaphase if the univalent chromosome divides in normal sequence.

There is evidence, presented below, that the bridges and fragments which occur spontaneously in meiosis of the *B. Trinii*×*maritimus* hybrid are the result of chromosome breakage and reunion such as that just described.

In the *B. Trinii*×*maritimus* hybrid a gradation from small constrictions to free fragments has been observed. Single chromatid fragments are numerous, and paired fragments and U-shaped fragments are also found. These appear to be identical with the fragments produced along with bridges by spontaneous chromosome breakage and reunion in Tradescantia (GILES 1940, 1941) and Tulipa (DARLINGTON and UPCOTT 1941) and by irradiation or chemical treatment in Tradescantia (SAX and MATHER 1939; SAX 1940; SWANSON 1942; DARLINGTON and KOLLER 1947), Chortophaga (CARLSON 1938, 1940) and Chorthippus (CREIGHTON 1941).

It has already been shown that in the *B. Trinii*×*maritimus* hybrid neither the univalent bridges observed at first anaphase nor the bridges found at second anaphase may be attributed to chiasma formation in heterozygous inversions. However, these bridges can result from chromosome breakage and reunion as follows: an isochromatid break in a univalent chromosome, followed by reunion of the broken ends of sister chromatids of the centric segment, or a chromosome break in a univalent, followed by splitting of the chromosome and reunion of the sister chromatids of the centric segment, forms a univalent loop chromosome at first anaphase (fig. 18 C). If such a univalent chromosome lags and divides precociously at first anaphase it forms a univalent bridge (figs. 5; 18 A); however, if this loop chromosome is included, undivided, at one pole at first telophase, it will divide to form a bridge at second anaphase. The observed increase in frequency of univalent bridges from early- and mid-anaphase to late anaphase in this hybrid may then be attributed to precocious division of this type of lagging univalent chromosome.

Anaphase bridges may also be formed in meiotic chromosomes from a combination of crossing-over and breakage and reunion, as WHITING (1945b) has described after irradiation of Habrobracon eggs. Thus an isochromatid break with reunion of the sister chromatids distal to a chiasma produces a bridge at first anaphase (fig. 2 C). Such an isochromatid break followed by reunion proximal to a chiasma will produce a loop chromosome at first division and a bridge at second anaphase (fig. 2 D); if the loop chromosome divides precociously at first anaphase a univalent bridge results.

The triangular configuration, observed at first anaphase (fig. 17), of three chromosomes connected by two bridges, could be produced by pairing and chiasma formation, breakage and reunion involving three univalents, or a combination of these methods of bridge formation. In order to produce such a configuration by means of chiasmata, the apical chromosome must form two chiasmata in its short arm, each between a sister chromatid and a chromatid of

another chromosome; the short arm of the apical chromosome, furthermore, must be inverted in relation to segments of the two chromosomes with which it is paired. This may be possible, but considering the low chiasma frequency it seems more probable that these two bridges were formed by asymmetrical chromatid interchange between three univalents, or by asymmetrical chromatid interchange between that portion of a univalent bearing the centromere and one chromatid of a bridge-forming true bivalent.

The dicentric ring chromosome observed at second anaphase (fig. 6) cannot be explained on the basis of chiasma formation. This configuration could, however, have been formed by an inter-arm deletion and subsequent division of the centric ring chromosome (fig. 1), or by an isochromatid or chromosome break and sister chromatid reunion in each arm of a univalent, followed by division of the centromere in second division.

On the basis of this evidence, it may be concluded that chromosome breakage and reunion occur spontaneously in meiosis of the *B. Trinii*×*maritimus* hybrid, and are responsible for the production of the numerous fragments observed, of the first anaphase univalent bridges, some of the first anaphase bivalent bridges, and, with the possible exception of a very few nondisjunction bridges, of all the second anaphase bridges.

The mitotic chromosomes of the mature *B. Trinii*×*maritimus* plants are presumed to have been normal, since the plants were sturdy, with well developed root systems. It is, however, possible that some fragmentation did occur in seedling root tips and that such aberrant cells were eliminated. Irregularities in root tip cells of Allium (SAX 1941; NICHOLS 1941) are eliminated or markedly decreased in mature plants, possibly because of differential survival of normal and aberrant cells. However, even if it did occur, fragmentation in seedling mitoses of the *B. Trinii*× *maritimus* hybrid must have been much less extensive than fragmentation in meiosis, which was observed in every anaphase cell, and it is possible that in this hybrid chromosome breakage occurs only in meiosis.

The exceptional first anaphase cell of the 96-chromosome allopolyploid (fig. 14), which exhibits numerous lagging chromosomes, bridges and fragments, may also be attributed to extensive chromosome breakage and reunion. Besides this cell, the attached fragments found in approximately 3 percent of the early first anaphase cells of the 96- and 98-chromosome allopolyploids are also attributed to chromosome breakage and reunion. The free fragments observed at first anaphase are difficult to interpret, since they may arise with the formation of a loop chromosome which will produce a second division bridge. However, in the 96-chromosome plant three free fragments were found in one early anaphase cell and a maximum of one bridge per cell at second anaphase; therefore it is probable that some of the free fragments observed in the allopolyploids are also the products of chromosome breakage. Including the exceptional cell with many bridges and fragments, it may be estimated that 3–5 percent of the early anaphase cells of the allopolyploids exhibit evidence of chromosome breakage and reunion.

Approximately 6 percent of the first anaphase cells of the allopolyploids

contain a bivalent bridge; no univalent bridges were found. Since the original allopolyploid was produced by means of somatic doubling of the hybrid, it could not have contained heterozygous inversions. However, since the allopolyploids reported here were the $C_3$ generation, it is possible that one or more inversions may have arisen in the two generations since its production. The bridge frequencies of *B. Trinii* and *B. maritimus* are not known; however, an occasional bridge has been observed in *B. Trinii*, and in *B. carinatus*, which is very closely related to *B. maritimus*, 7–9 percent of the first anaphase cells contained a bridge (STEBBINS and TOBGY 1944), suggesting that inversions take place occasionally in the parent species. Because of the relatively regular pairing and chiasma frequency, and because the allopolyploids studied were the $C_3$ generation, it is not possible to determine whether any of the bridges reported above is the result of breakage and reunion.

The chiasma frequency in these allopolyploids is high enough so that second division bridges may arise by means of crossing over in heterozygous inversions. It is difficult, however, to account for the difference in bridge frequencies at second division between the 96- and 98-chromosome plants. This may be an indication that some of the bridges in the 96-chromosome plant are due to breakage and reunion, as in the hybrid; however, there is no evidence, except for the one exceptional cell, of the occurrence of more fragmentation in the 96- than in the 98-chromosome plant. As the number of second anaphase cells studied in the 96-chromosome plant was very small, the difference in bridge frequency may not be actually significant.

Thus it may be stated that with the exception of one cell, the *B. maritimus-Trinii* allopolyploids which were studied are relatively regular in meiosis, with only a few cells giving evidence, on a small scale, of the fragmentation and reunion which occur so widely in the hybrid.

It should be noted that in the population ($C_2$) grown from selfed seed of the original allopolyploid, the seed set ranged from 0–80 percent; seed collected from the 12 most fertile of these plants formed the population studied ($C_3$) (STEBBINS and WALTERS, in press). Thus there is the possibility that extensive fragmentation and reunion may have occurred in some of the infertile plants of the $C_2$ generation, and that selection of seed from the most fertile plants may have resulted in the relatively regular meiosis observed in the $C_3$ population.

## DISCUSSION

It is expected that a small amount of chromosome pairing may be found at metaphase in the *B. Trinii*×*maritimus* hybrid; there is some evidence (STEBBINS, TOBGY, and HARLAN 1944; STEBBINS unpub.) that *B. Trinii* has one genome partly homologous with a genome of *B. carinatus*, which is closely related to *B. maritimus*. However, the occurrence of extensive chromosome breakage and reunion in the *B. Trinii*×*maritimus* hybrid has been established, and it will be shown below that it probably occurs in early prophase. Therefore it is possible that the bivalents and trivalents observed at metaphase in this hybrid may be the result of chiasma formation, or breakage and reunion, or both.

Bivalents such as those shown in figure 3, a-l may result from partial or complete terminalization of one chiasma in a pair of partly homologous chromosomes (fig. 2 A). In certain of these bivalents it appears as if there is tension between the centromeres and the terminal regions of the paired arms (fig. 3, i-l). DARLINGTON (1937) has presented evidence that chiasmata do not terminalize past non-homologous regions, and also that at metaphase the two centromeres of a bivalent repel each other actively. Thus if an interstitial chiasma is present at metaphase in a bivalent with non-homologous regions distal to the chiasma, the repulsion of the two centromeres causes the chromosome segments between the centromeres and the chiasma (or first chiasma if there are more than one) to be drawn out into a fine thread, while the other parts of the bivalent show no tension (*loc. cit.*). Such an explanation may account for configurations such as those shown in figure 3, k-l; however, in order to explain bivalents i-j, it must further be assumed that both arms between the centromeres and the interstitial chiasma are not under equal tension or do not respond with a similar amount of stretching.

The small opening in the bivalents shown in figure 3, e, f, probably indicates the presence of a chiasma. Bivalents such as those shown in figure 3, a–d, g–l may also be formed by asymmetrical chromatid interchange between two non-homologous chromosomes (fig. 2 B). Since the fragments generally remain in contact with the broken chromosome throughout metaphase, they may remain associated with the fused region. If the breaks were very close to the chromosome ends, so that the fragments were minute, configurations such as those shown in figure 3, a–d could be formed; if the breaks were far enough from the ends of the chromosome arms so that the fragments were relatively large, configurations such as those shown in figure 3, g–k could be produced. There is also the possibility of bivalent formation through asymmetrical chromosome interchange; however, such a chromosome interchange should produce a double chromosome bridge at first anaphase and none has been observed.

Unless it is assumed that breakage and reunion occur only in univalents in this hybrid, some of the metaphase bivalents and trivalents may result from a combination of chiasma formation with breakage and reunion. If there is an isochromatid or chromosome break followed by sister chromatid reunion proximal to a chiasma (fig. 2 D) the metaphase bivalent actually consists of two separate structures. There could be no terminalization of the chiasma between the unbroken chromosome and the fragment, since any movement of the unbroken chromosome would carry the fragment with it. The configuration shown in figure 3, m may have been produced in this way; if a chromatin strand were to connect the two structures, bivalents such as those shown in figure 3, i–j could result. With an isochromatid or chromosome break and sister chromatid reunion distal to a chiasma (fig. 2 C), configurations such as those shown in figure 3, d, g, h, k could result; however, it may be observed in figure 2 that it is not possible to distinguish at metaphase between bivalents produced by breakage and reunion distal to a chiasma, those produced by asymmetrical chromatid interchange, and those produced by one chiasma. It should be noted

that configurations similar to those produced by a combination of chiasma formation and breakage and reunion can be formed from univalents, without the production of chiasmata; if, in each case, the chiasma is replaced by a corresponding asymmetrical chromatid interchange, the end result is the same (see THODAY 1942, fig. 3).

The trivalents shown in figure 3, o–r, also could be formed either by pairing and chiasma formation in homologous segments of three chromosomes or by asymmetrical chromatid or chromosome interchange between three univalents. The chain trivalent (fig. 3, o–p) is produced if each arm of one chromosome is paired with a different chromosome, and a chiasma formed in each paired arm. Or, it could be formed if two chromosomes were united by means of an asymmetrical chromatid interchange and one of these chromatids was united at its opposite end with a third chromosome in the same way (fig. 1). Asymmetrical chromosome interchange between three chromosomes is considered unlikely owing to the absence of double bridges at first anaphase.

The Y-trivalent (fig. 3, q-s) is produced if each of two segments of one chromosome arm is paired with a different chromosome and a chiasma is formed in each paired segment. The small opening in the trivalent shown in figure 3, s, probably indicates the presence of a chiasma. Or, configurations such as those illustrated in figure 3, q, r, could result from an *asymmetrical isochromatid-chromatid interchange*; *i.e.*, after an isochromatid break, one centric chromatid is fused with a centric chromatid of a second chromosome (which has suffered either an isochromatid or chromatid break), and its sister chromatid fused with a centric chromatid of a third chromosome (fig. 1). Such a breakage and reunion trivalent should produce a triangular structure at anaphase, each of two chromosomes attached to a third chromosome by a single bridge (fig. 17).

The determination of the stage during which breakage takes place in meiosis of the *B. Trinii*×*maritimus* hybrid can be attempted from indirect evidence. Pachytene chromosomes generally exhibit no visible evidence of breakage, but breakage may nevertheless have occurred. While BISHOP (1942) has observed breaks in pachytene and diakinesis chromosomes of Melanoplus six minutes after irradiation and in diakinesis chromosomes of Chortophaga three minutes after irradiation, GOODSPEED (1929) has found that Nicotiana chromosomes irradiated in early meiotic prophase generally appear to be morphologically unchanged until anaphase, when fragmentation becomes visible; he describes such chromosomes as "internally but not visibly fragmented."

The observation of chromosome stickiness has sometimes been related to the stage of meiosis or mitosis at which irradiation occurred. Generally stickiness and fragmentation are not found in the same cells, but there is the possibility of such a combination of physiological and structural effects (LEA 1947). A small proportion of sticky cells has been observed in all stages of meiosis in the *B. Trinii*×*maritimus* hybrid (and in some metaphase cells of the 96-chromosome allopolyploid), and two exceptional slides, containing an unusual range of stages, had sticky prophase cells in which some free fragments were observed; no fragments were observed in metaphase cells on these slides. However, it cannot be determined whether the stickiness in this hybrid is

related to the phenomena of breakage and reunion, since interspecific grass hybrids frequently have sticky meiotic chromosomes, and this condition may also be influenced by environmental conditions.

Since the spontaneous and X-ray induced aberrations seem to be identical, examination of the behavior of irradiated chromosomes in other organisms may give some indication as to the time of spontaneous breakage in this hybrid. LEA (1947) states that for the production of structural changes it is necessary to irradiate during the resting stage or early prophase; irradiation during metaphase and anaphase leads to aberrations observed during the following division. WHITING (1945 a, b) has found that in Habrobracon irradiation of diplotene results in bridges and fragments at first or second anaphase or both, whereas irradiation at first metaphase results in acentric fragments but no bridges at first anaphase and bridges at second anaphase. In Trillium (SPARROW 1944) irradiation of prophase produces bridges and fragments at first anaphase, but irradiation of metaphase-anaphase produces fragments at the first microspore mitosis. On this basis, it seems probable, since bridges and fragments were observed at first anaphase in the *B. Trinii*×*maritimus* hybrid, that the breakage took place previous to metaphase.

Irradiation of the resting stage generally seems to produce a high proportion of chromosome breaks and reunions, whereas irradiation of prophase generally seems to produce a high proportion of chromatid breaks and reunions (RILEY 1936; SAX 1940; NICHOLS 1941; GILES 1941). The single, U-shaped, and paired fragments found in the *B. Trinii*×*maritimus* hybrid have been described previously in other materials and attributed to both chromosome and chromatid (including isochromatid) breakage. Single chromatid fragments generally arise by means of a chromatid break, as in Tradescantia (SAX 1940; GILES 1941) or less often following an isochromatid break with restitution of one chromatid (CATCHESIDE 1948). The U-shaped fragment is generally produced by an isochromatid break followed by fusion of the broken ends of the acentric fragment, as in Tradescantia (GILES 1940; SAX and MATHER 1939), Chorthippus (CREIGHTON 1941), and Chortophaga (CARLSON 1941), but may also be produced by a chromosome break followed by splitting of the fragment and fusion of its two broken ends, as in Chortophaga (CARLSON 1938). The paired fragments observed in Tulipa (DARLINGTON and UPCOTT 1941) and Tradescantia (GILES 1940, 1941; SAX and MATHER 1939) have been attributed to a chromosome break followed by division of the chromosome and fragment; however, paired fragments observed in Chorthippus (CREIGHTON 1941) are produced by isochromatid breaks.

Chromosome rearrangements comparable to the univalent and bivalent bridges found in this hybrid have also been observed in other organisms and attributed to either chromatid or chromosome breakage. A mitotic anaphase bridge, comparable to a univalent bridge, is most often produced by an isochromatid break with sister chromatid reunion, as in Tradescantia (SAX and MATHER 1939), but also by a chromosome break followed by division and reunion of sister chromatids, as in Chortophaga (CARLSON 1941). A mitotic anaphase bridge comparable to a bivalent bridge is most frequently produced

by an asymmetrical chromatid interchange following a chromatid or isochromatid break, as in Tradescantia (SAX and MATHER 1939; THODAY 1942; CATCHESIDE, LEA, and THODAY 1946), but perhaps it may also result from asymmetrical chromatid interchange following a chromosome break and division (cf. DARLINGTON and LA COUR 1945; DARLINGTON and KOLLER 1947).

However, chromosome breaks are generally followed by some asymmetrical chromosome interchanges (SAX 1940, 1941; CARLSON 1941), and after the chromosome becomes functionally double such an interchange should produce a double bridge between two chromosomes at anaphase (fig. 1). It has been stated above that no double bridge was observed in this hybrid. Although the presence of single bridges does not eliminate the possibility of chromosome breaks, the complete absence of double bridges does indicate their absence or infrequent occurrence.

On the basis of the above indirect evidence, it seems probable that in the *B. Trinii*×*maritimus* hybrid, the majority of the breakage and reunion took place in prophase, after the chromosomes had become effectively double, and therefore that the majority of the breaks are isochromatid and chromatid breaks which are followed by chromatid reunions and interchanges.

Besides producing chromosome breakage, irradiation and chemical treatment of chromosomes is known to have an effect on the centromeres. The inhibition of mitosis by X-rays and the induction of diplochromosomes are attributed to an action of X-rays on the centromeres (CATCHESIDE 1948); stretched centromeres, mis-division, and irregular behavior in metaphase and anaphase are attributed to the action of mustard gas on the centromeres (DARLINGTON and KOLLER 1947). Stretched centromeres are also occasionally observed in the *B. Trinii*×*maritimus* hybrid (fig. 18 G).

There is a possibility that a configuration observed in meiosis of this hybrid may also be attributed to a centromere effect which accompanies the spontaneous chromosome breakage; the loop configurations found in some telophase cells may result from the occasional abnormal behavior of the centromere of a loop chromosome, such as would ordinarily form a bridge at first or second anaphase. Each of the two loops observed at first telophase has two arms, each of which appears to contain a centromere, and these lie very close together (figs. 7 A, B; 13 A, B). In the majority of second telophase loops the arms of the loop are hidden from view in the nucleus; however, where they can be seen (figs. 11 A, B) each arm appears to have a centromere, and the two centromeres lie close together. With two exceptions the loop configurations were found at second telophase, and it has previously been shown that second division bridges result from division and movement of the centromeres of a loop chromosome to separate poles; the two loop configurations found at first telophase are probably formed from precociously dividing loop chromosomes. Therefore the suggestion is made that occasionally after the centromere of a loop chromosome divides, the two daughter centromeres do not separate; perhaps the repulsion between them (DARLINGTON 1937) is not operative or effective or is at least diminished. However, it is not possible at present to explain the elongation of the loop portion of this configuration (which generally appears to

be approximately as long as bridges observed in the same cells) since the centromeres are not moving towards opposite poles.

Very little is known about the method of spontaneous chromosome breakage. GILES (1940) has found that the rate of spontaneous breakage in Tradescantia microspore mitosis cannot be accounted for in terms of natural radiation. The great difference in amount of breakage in meiosis of the *B. Trinii*×*maritimus* hybrid and its allopolyploid, both grown in the same locality, cannot be explained on this basis. GILES (1940) suggests that torsional strains may be reponsible for the spontaneous breaks in Tradescantia.

HAGA (1937) suggests that the breakage in meiosis of Kinugasa is caused by environmental factors. However, there is evidence that the spontaneous chromosome breakage reported in mitosis has a genetic basis. NICHOLS (1941) found variation in frequency of aberrations in Allium root tips grown from seed of the same age, and suggested that the variation was due to inherent differences in susceptibility to spontaneous chromosome breakage. PLOTNIKOWA (1932) found considerable variation in frequency of bridges and fragments in root tips in certain Triticum×Secale populations. GILES (1940) found variation in the amount of breakage and reunion in $F_2$ segregates of a Tradescantia species hybrid, and further that the frequency of spontaneous breaks was about three times as high in the hybrid as in the pure species. He suggests (citing PLOTNIKOWA) that the higher frequency of chromosome breaks following hybridization may be due to recombination of genetic factors influencing chromosome coiling. Furthermore, GILES (1941) finds that there is almost six times as much breakage in triploid interspecific Tradescantia hybrids as in diploid hybrids, and suggests that the increase in the triploids is due to genetic unbalance.

It may be suggested that interaction of the genomes of *B. Trinii* and *B. maritimus* brings about chromosome breakage in meiosis in this hybrid. The question then arises: Why does the allopolyploid, which has the same genetic constitution as the $F_1$ hybrid in double quantity, exhibit relatively regular meiosis? It has been pointed out above that the allopolyploid population studied represents a selected population, since it was grown from seed collected from the most fertile plants of the preceding generation, and therefore that there is the possibility of some elimination of meiotic irregularity through this selection. However, even if this had occurred, it would still be necessary to account for the lack of extensive breakage in those allopolyploids from which seed was collected.

Aside from the fragmentation, the most obvious difference between meiosis in the hybrid and allopolyploid is in the number of univalents characteristic of each. In the hybrid, with 49 chromosomes, 37–49 univalents have been found at metaphase, and in the allopolyploid, with about 98 chromosomes, 0–10 univalents have been observed at this stage. It may then be suggested that the combination of *Trinii* with *maritimus* genomes, as found in the hybrid and allopolyploid, sets in motion some process leading to chromosome breakage, but that the process is effective only with univalent chromosomes. On this basis the single markedly aberrant anaphase of the 96-chromosome allopoly-

ploid shown in figure 14 would result from a rare case of nearly complete lack of pairing. It is not possible to determine whether, in the hybrid, univalents alone are affected, since an apparent metaphase bivalent or trivalent with a constriction (fig. 15) may actually consist of univalents held together by interchanges.

It is true that in both the hybrid and allopolyploid there are many more univalents at metaphase than there are anaphase chromosomes showing evidence of breakage. However, it has been shown that only a small proportion of the chromosome or chromatid breaks produced in Tradescantia result in visible breaks or structural rearrangements (SAX 1940; LEA and CATCHESIDE 1942; CATCHESIDE, LEA, and THODAY 1946). It is probably safe to assume that this is also true in the *B. Trinii*×*maritimus* hybrid and therefore that the aberrations observed at anaphase are not necessarily disproportionate to the number of univalents found at metaphase.

Experiments concerning this question are now in progress.

## SUMMARY

*Bromus Trinii* (2n=42) and *B. maritimus* (2n=56) exhibit completely regular meiotic divisions. Both species have more than 90 percent stainable pollen and are highly fertile.

The hybrid, *B. Trinii*×*maritimus* (2n=49) is more vigorous than the parent species, but exhibits no stainable pollen and is completely sterile. At first metaphase there are approximately 4 bivalents and trivalents per cell; 37–49 univalents have been found. The chiasma frequency is very low, 0.56 per chromosome in bivalents and trivalents. Free fragments are rare but constrictions in the chromosomes are frequently found. At first anaphase bridges are observed (approximately 1.1 per cell), including some univalent bridges, and numerous fragments appear in every cell; as many as 28 fragments have been found in a single cell. At second anaphase there is an average of 3.5 bridges per dyad, and a range of 1–8 bridges per dyad. Since there is very little pairing at metaphase, and never two chiasmata in one arm of a bivalent or trivalent, these second division bridges cannot have been produced by crossing over in heterozygous inversions. The presence of these bridges at second anaphase, the formation of univalent bridges at first anaphase, and the numerous fragments have led to the conclusion that chromosome breakage and reunion take place in meiosis of this hybrid, apparently identical with that reported to follow irradiation of other species and to have occurred spontaneously in mitosis of other species. Bridges are formed when chromosome breakage is followed by reunion of chromatids of centric segments in such a way that dicentric chromatids are produced.

Because no double chromosome bridges have been found, it is suggested that most or all of the chromosome breakage took place in prophase of this hybrid, after the chromosomes had become effectively double, and therefore that the majority of the breaks are isochromatid and chromatid breaks which are followed by chromatid reunions and interchanges. If the breakage and reunion took place in prophase, then it is probable that some of the bivalents

and trivalents observed at metaphase have been formed by breakage and reunion of non-homologous univalent chromosomes or by a combination of breakage and reunion and chiasma formation, and that the true pairing and chiasma frequency at metaphase is even lower than that reported above.

The allopolyploid *B. maritimus-Trinii* (2n=98; however, a range of 96–100 was found) has about 80 percent stainable pollen and is highly fertile. Meiosis is regular, with few multivalents, none higher than a quadrivalent, and relatively few univalents (0–10). Only one first anaphase cell, observed in the 96-chromosome allopolyploid, exhibited several bridges and numerous fragments and precociously divided chromosomes. Aside from this, 3–4 percent of the anaphase cells exhibit evidence of a limited amount of breakage. The allopolyploids studied were grown from seed collected from the 12 most fertile plants of the preceding generation, in which seed set ranged from 0–80 percent. Thus there is the possibility that selection has resulted in the relatively regular meiosis observed. Nevertheless it would still be necessary to account for the lack of extensive breakage in the most fertile allopolyploids from which seed was collected. To explain the marked difference in the extent of breakage and reunion in the hybrid and allopolyploid, it is suggested that the interaction of *Trinii* and *maritimus* genomes in the hybrid and allopolyploid sets in motion the processes leading to chromosome breakage, but that these are effective only with univalent chromosomes. Experiments for the purpose of testing this hypothesis are in progress.

ACKNOWLEDGEMENTS

I wish to thank DR. G. LEDYARD STEBBINS, JR. for providing me with the collected material of the hybrid *B. Trinii*×*maritimus* from which this study was made as well as for a critical reading of the manuscript. I also wish to thank DR. R. E. CLAUSEN and DR. D. G. CATCHESIDE for critical reading of the manuscript, and DR. JAMES L. WALTERS for assistance in taking some of the photographs and in preparation of the manuscript.

LITERATURE CITED

AKEMINE, T., 1940 Abnormal meiosis in genus Hosta (A preliminary note). Jap. J. Genet. **16:** 13–16.

BISHOP, D. W., 1942 Cytological demonstrations of chromosome breaks soon after X-radiation. Genetics **27:** 132.

CARLSON, J. G., 1938 Some effects of X-radiation on the neuroblast chromosomes of the grasshopper *Chortophaga viridifasciata*. Genetics **23:** 596–609.

1941 Effects of X-radiation on grasshopper chromosomes. Cold Spring Harbor Symp. Quant. Biol. **9:** 104–111.

CATCHESIDE, D. G., 1945 Effects of ionizing radiations on chromosomes. Biol. Rev. **20:** 14–28.

1948 Genetic effects of radiations. Advances in Genetics, **2:** 271–358.

CATCHESIDE, D. G., D. E. LEA, and J. M. THODAY, 1946 Types of chromosome structural change induced by the irradiation of Tradescantia microspores. J. Genet. **47:** 113–136.

CREIGHTON, M., 1941 The effects of X-rays on mitotic and meiotic chromosomes and cell division in *Chorthippus longicornis* (Orthoptera). J. exp. Zool. **87:** 347–369.

DARLINGTON, C. D., 1937 Recent Advances in Cytology, 2nd ed. XVI+671 pp. London: J. and A. Churchill, Ltd.

DARLINGTON, C. D., and P. C. KOLLER, 1947 The chemical breakage of chromosomes. Heredity **1:** 187–221.

DARLINGTON, C. D., and L. F. LA COUR, 1945 Chromosome breakage and the nucleic acid cycle. J. Genet. **46:** 180–267.

DARLINGTON, C. D., and M. B. UPCOTT, 1941 Spontaneous chromosome change. J. Genet. **41:** 297–338.

EMSWELLER, S. L., and P. BRIERLEY, 1943 Effects of high temperature on metaphase pairing in *Lilium longiflorum*. Bot. Gaz. **105:** 49–57.

GILES, N., 1940 Spontaneous chromosome aberrations in Tradescantia. Genetics **25:** 69–87.
1941 Spontaneous chromosome aberrations in triploid Tradescantia hybrids. Genetics **26:** 632–649.

GOODSPEED, T. H., 1929 The effects of X-rays and radium on species of the genus Nicotiana. J. Hered. **20:** 243–259.

HAGA, T., 1937 Chromosome complement of *Kinugasa japonica* with special reference to its origin and behavior. Cytologia **8:** 137–141.

KIHARA, H., 1931 Genomanalyse bei Triticum und Aegilops. II. Aegilotricum und *Aegilops cylindrica*. Cytologia **2:** 106–156.

KNOWLES, P. F., 1944 Interspecific hybridization of Bromus. Genetics **29:** 128–140.

LEA, D. E., 1947 Actions of Radiations on Living Cells. XII+402 pp. New York City: The Macmillan Co.

LEA, D. E., and D. G. CATCHESIDE, 1942 The mechanism of the induction by radiation of chromosome aberrations in Tradescantia. J. Genet. **44:** 216–245.

MARSHAK, A., 1935 The effect of X-rays on chromosomes in different stages of meiosis. J. gen. Physiol. **19:** 179–198.
1939 A comparison of the sensitivity of mitotic and meiotic chromosomes of *Vicia faba* and its bearing on theories of crossing over. Proc. nat. Acad. Sci. **25:** 510–516.

MARSHAK, A., and W. S. MALLOCH, 1942 The effect of fast neutrons on chromosomes in meiosis and its bearing upon pachytene pairing. Genetics **27:** 576–583.

NICHOLS, C., 1941 Spontaneous chromosome aberrations in Allium. Genetics **26:** 89–100.

PLOTNIKOWA, T. W., 1932 Zytologische Untersuchung der Weizen-Roggen Bastarde. I. Abnormale Kernteilung in somatischen Zellen. Planta **16:** 174–177.

RICHARDSON, M. M., 1936 Structural hybridity in *Lilium Martagon album*×*L. Hansonii*. J. Genet. **32:** 411–449.

RILEY, H. P., 1936 The effect of X-rays on the chromosomes of *Tradescantia gigantea*. Cytologia **7:** 131–142.

SAX, K., 1938 Chromosome aberrations induced by X-rays. Genetics **23:** 494–516.
1940 An analysis of X-ray induced chromosomal aberrations in Tradescantia. Genetics **25:** 41–68.
1941 The behavior of X-ray induced chromosomal aberrations in Allium root tip cells. Genetics **26:** 418–425.

SAX, K., and K. MATHER, 1939 An X-ray analysis of progressive chromosome splitting. J. Genet. **37:** 483–490.

SPARROW, A. H., 1944 X-ray sensitivity changes in meiotic chromosomes and the nucleic acid cycle. Proc. nat. Acad. Sci. **30:** 147–155.

STEBBINS, G. L., JR., 1949 The evolutionary significance of natural and artificial polyploids in the family Gramineae. Hereditas, Suppl. Vol.: 461–485.

STEBBINS, G. L., JR., and H. A. TOBGY, 1944 The cytogenetics of hybrids in Bromus. I. Hybrids within the section Ceratochloa. Amer. J. Bot. **31:** 1–11.

STEBBINS, G. L., JR., and M. S. WALTERS, 1950 The evolutionary significance of two synthetic allopolyploid species of Bromus. Portugaliae. Acta Biologica (in press).

STEBBINS, G. L., JR., H. A. TOBGY, and J. R. HARLAN, 1944 The cytogenetics of hybrids in Bromus II. *Bromus carinatus* and *Bromus arizonicus*. Proc. Calif. Acad. Sci. **25:** 307–322.

STONE, L. H. A., 1933 The effect of X-radiation on the meiotic and mitotic divisions of certain plants. Ann. Bot. **47:** 815–826.

SWANSON, C. P., 1942 The effects of ultra violet and X-ray treatment on the pollen tube chromosomes of Tradescantia. Genetics **27**: 491–503.

THODAY, J. M., 1942 The effects of ionizing radiations on the chromosomes of *Tradescantia bracteata*. A comparison between neutrons and X-rays. J. Genet. **43**: 189–210.

WHITING, A. R., 1945a Effects of X-rays on hatchability and on chromosomes of Habrobracon eggs treated in first meiotic prophase and metaphase. Amer. Nat. **79**: 193–227.

1945b Dominant lethality and correlated chromosome effects in Habrobracon eggs X-rayed in diplotene and in late metaphase I. Biol. Bull. **89**: 61–71.

# CONSEQUENCES OF CROSSING OVER IN OÖGONIAL CELLS

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Received April 11, 1949

IN TESTCROSSES it is commonly supposed that each recombination offspring owes its phenotype to chiasmata in a single primary oöcyte which gives rise to no other offspring. This assumption should be questioned in view of the demonstration that crossing over may be induced by X rays in oögonial cells of *Drosophila melanogaster* (WHITTINGHILL 1938). Irradiation is known to increase the percentage of recombinations in females in some manner, and in males the same agent is able to induce crossovers which originate largely or exclusively in spermatogonial cells. It is therefore appropriate to consider the various possible effects of crossing over in oögonial cells upon the whole picture of recombinations as found in testcrosses. Any oögonial crossing over would make the basic assumption of a linear relationship between chiasma frequencies and recombination partially incorrect.[1] Hence conclusions regarding the quantitative effects of various external and interchromosomal agents upon the process of crossing over may be subject to large errors usually unsuspected.

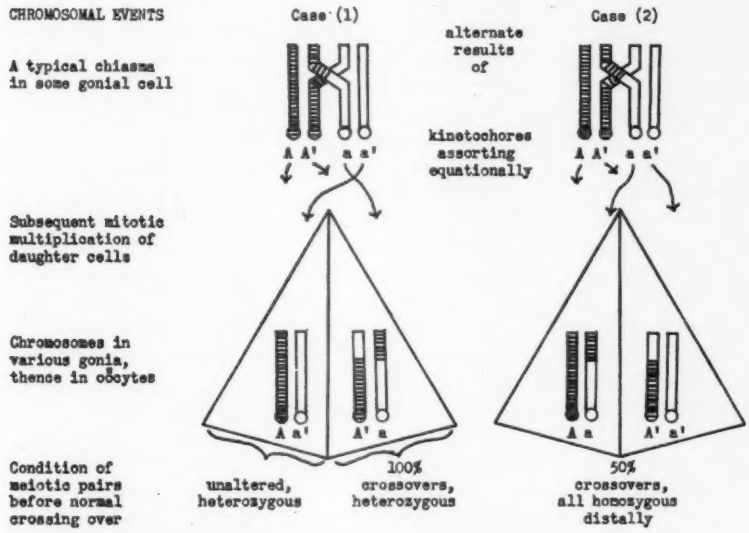
Large changes in the frequency of certain classes of progeny will result from one crossing over in an early gonial cell, and smaller effects will follow an exchange in a later cell division. This lack of a constant proportionality between crossover offspring and inferred chiasmata, so clearly demonstrated by this author in males (1937) and revealed in *c3G* females (1938), may be reasonably applied also to ordinary heterozygous females. This variable relationship may be visualized in figure 1 by imagining that the pyramids shown there may be of various sizes representing the different numbers of cells formed by mitosis after any gonial exchange and before the initiation of meiosis in the descendant cells. The methods of detecting oögonial crossing over through the smoke screen of more numerous meiotic exchanges will vary depending on whether the oögonial exchanges have occurred early or late.

The essential ideas of this paper have been incorporated into the diagrams and tabular material of figure 1. It shows the kinds of effects produced in a testcross by a single gonial crossing over depending on the alternate ways in which the four strands of the tetrad may be distributed to daughter cells. STERN (1936) in an extensive investigation of segregation in somatic tissues has shown that anaphase separations of the two kinds figured may be expected following crossing over during mitosis. Those of type (2) would produce segre-

[1] Very recently COOPER (1949) has challenged this classic tenet in another way by showing that chiasmata may occur without genetic crossing over. For the sake of brevity this paper will retain the older usage, *i.e.*, "chiasma" here means "one occurrence of the process of crossing over" regardless of how many crossover offspring may be produced by it.

gation (qualitative reduction) of the formerly heterozygous loci distal to the inferred chiasma resulting in somatic spots of recessive phenotype, but anaphase separations of type (1) would produce no somatic segregation because both daughter cells would remain heterozygous and of dominant phenotype. In the gonial line, however, either type of segregation of chromatids would have genetic consequences as indicated in figure 1.

The main thing about oögonial exchange is that it prevents all cells from being identical when meiosis begins. Four different starting conditions are possible, as diagrammed, and these will be further altered, detectably or not,




RESULTS OF TESTCROSS

| | | | |
|---|---|---|---|
| Recombination in region of any gonial exchange | usual meiotic value | increased* in one region | increased* in one region |
| Recombination distal to above | " | meiotic value | decreased* |
| Recombination proximal to above | " | " | meiotic value |
| Double & multiple recombinations | " | increased* & non-random | reduced distally, non-random |
| Interference | " | reduced* | reduced proximally |

* Phenomena already reported by various workers and here interrelated. See text.

FIGURE 1.—Effects of crossing over in an early gonial cell upon recombination phenotypes.

by the usual meiotic exchanges. The genetic consequences of random meiotic crossing over acting uniformly upon the different synaptic mates are tabulated under each kind of pairing situation. It is of special interest that most of the changes deduced in the figure have already been separately reported by several experimenters to be cited in turn. The explanation of these independent observations may be partly or wholly the consequences of relatively few oögonial chiasmata. The figure considers one typical chiasma, but it should be remembered that in other parallel germ lines effective chiasmata may occur in various regions along the chromosome and also that they may produce cumulative results. Both individual and mass effects will be elaborated.

The accurate detection of gonial crossing over in either sex requires that the data of the various families be scrutinized for inconsistencies before the totals for a whole experiment are made. In fact within the family irregularities may appear if egg-laying is prolonged through many successive cultures. Six ways in which families may be inhomogeneous will now be described. The first three could indicate any individual early gonial crossing over with many progeny derived from it. The last three could reveal the combined action of later gonial exchanges, particularly if they were numerous in occurrence. The six phenomena exhibit some interdependence.

First, an excess of crossovers from one region in one family or subfamily may point beyond meiotic exchanges to a single crossing over in that region in an early gonial cell. An excess of crossovers could result after either chance of segregation, (1) or (2). It is rather well established that spotty distributions of crossovers do actually exist particularly near spindle attachments.

Second, an imbalance of complementary crossover classes independent of viability differences may result following segregation (2), provided that the segregants pass into gonial cells which divide at unequal rates. This would be a departure from the more usual multiplication shown in figure 1, where equal sides of a pyramid symbolize equal reproduction of the two kinds of daughter cells. Gross inequalities of this kind have been found by the writer and others following high temperature treatment and after irradiation of male heterozygotes. Hence they may be occurring also in females. If the complementary crossovers are unequally propagated, it is a corollary that a certain non-crossover will likewise be unequally reproduced, namely, the one with the homologous, non-identical kinetochore. Any difference of this sort would usually be diluted by the large numbers of non-crossovers from all other sources; therefore, the favoring of one non-crossover would show only in extreme cases. FRIESEN (1936) has reported some families from X-rayed males with total recombinations of 25 percent, 27 percent and 31 percent which had the complementary crossover classes in ratios of about 2:1, 5:1 and 8:1, respectively. His published data did not show separately the two classes of non-crossovers, which would be of particular interest in these three families.

Third, a reduction of recombination values in regions distal to some region showing an increase would indicate that partial homozygosis had been produced by segregation of type (2). This homozygosis would cause a certain proportion of the subsequent meiotic crossing over to produce no detectable

recombinants of distal genes. Such a depression of the distal recombination frequency would be less than the elevation of the proximal region of exchange, because many of the meiotic tetrads would have given rise to non-crossover strands anyway. Although the effect of distal homozygosis caused by one gonial exchange will be rather small, the effect of several should add up to a conspicuous phenomenon, if crossing over has occurred in many oögonial cells. This and other cumulative effects comprise the remaining three ways of detecting whether oögonial crossing over has taken place under one set of conditions more than under another.

Fourth, progressive distal homozygosis may be expected to result in the masking of some of the subsequent meiotic (and rarely other gonial) exchanges. Homozygosis of a locus before meiosis will be produced by some 50 percent of the gonial exchanges which form between it and the spindle attachment. Loci which are farther from the spindle attachment will become homozygous more often than those which are closer. A similar rise in homozygosis of the more distal genes has been described by BEADLE and EMERSON (1935) in attached-X chromosomes, where, as in mitosis, two chromatids from a tetrad may be recovered. Hence the strands which physically cross over in the region of the spindle attachment, whether during mitotic or meiotic divisions, have the best chance of being represented as crossover offspring. It is a fact that the greatest increases in recombination are at the spindle attachment region following the use of any of the presently known inducing agents except partial starvation of larvae. The regions more removed from the spindle attachment will be less likely to show their usual number of meiotic crossovers, and these losses of normal recombinations should be most pronounced in the most distal region. Hence the induced recombinations formed distally in oögonial cells may not raise crossover values above normal because they will be added to classes already diminished.

Many observed facts fit this scheme. The first known effect of X rays upon crossing over was a non-inherited decrease in recombination in the distal part of the sex chromosomes of *D. melanogaster*, reported by MAVOR (1923), although he soon found that irradiation increased recombination near the spindle region of the second chromosome pair. SCHULTZ and REDFIELD (1932, 1933) observed that interchromosomal effects upon recombination were greatest in the spindle region, least distally. Sometimes the enhancing agent acts negatively in the farthest region, and KIKKAWA (1934) has pointed out the rather frequent finding of what he has termed "proximal increases and compensatory distal decreases." In experiments using a nitrogen mustard, the present writer (1948b) has found similar results. Treated rucuca heterozygotes of *D. melanogaster* had fewer recombinations in the two ends of the chromosome than untreated sisters had, and elsewhere they gave above normal values which increased regularly toward the spindle attachment to a pronounced peak there, 4½ times the control value. Less extreme but similarly graded departures from controls appeared in rucuca heterozygotes as an interchromosomal effect of 7 out of 8 X-chromosome inversions tested by STEINBERG and FRASER (1944) and showing any increases. An explanation of the tendency to compensatory

distal decreases is inherent in the present hypothesis. If numerous chiasmata have formed crossovers in gonial cells at any time, then there may be a net scarcity of the more distal recombinations without recognizable clusters of the various proximal crossovers responsible. Although each crossover adds to the recombination in its own region, half of them subtract from two, three or all distal regions.

In addition to the better transmission of recombinations formed in the spindle attachment region, an actual concentration of gonial crossing over in that vicinity may further increase the departure of testcross data from normal. Although it does not seem feasible to study directly the distribution of gonial crossing over in females, a spermatogonial map of chromosome 3 has been made by the writer (1948a) using data from irradiated males of *D. melanogaster*. Forty-nine percent of the third chromosome exchanges occurred in the short scarlet-curled region of males, which is 6 units in length on the female map, whereas 51 percent were elsewhere in 94.7 standard units of length. If the spermatogonial crossing over map applies also to the gonial cells of females, that distribution combined with distal homozygosis would account for the great peak of relative increases in recombination at the spindle attachment region of this chromosome.

Fifth, reduced interference should also result from gonial crossing over. Since gonial and meiotic chiasmata occur in different cell generations, there can be no mechanical interference between them in chiasma formation. Hence double and multiple recombinations will increase. Interference will appear to be reduced between the spindle attachment and the locus of the oögonial crossing over. Distal to any gonial exchange interference will appear to be affected in opposite ways depending upon the kind of mitotic segregation which has occurred. When the two crossover chromosomes go together, (1) in figure 1, any meiotic crossing over distally can be detected when it occurs, so such data will point to lessened interference. SCHULTZ and REDFIELD (1933) have observed a general reduction. STEINBERG and FRASER found it in some experiments but not in others. When segregation of kind (2) occurs in mitosis fewer meiotic crossovers for distal regions will be found. Thus when heterozygosis is retained until meiosis, coincidence will rise toward 1, and when heterozygosis is lost coincidence values will be depressed toward 0 for certain distal regions. It may be concluded from the above reasoning that coincidence even in massed data may appear to be increased proximally more than distally by the action of oögonial crossing over.

Sixth, an excess of any of the rarely occurring classes of crossovers may point to oögonial crossing over. Rare crossovers may be multiple, double or single crossovers, and they should be defined within each family in terms of the recombination measured there. Non-random distribution of rare crossovers has been found by SEREBROVSKY (1927), BRIDGES (personal communication), and others including the writer. Their interpretation in terms of the gonial hypothesis or some other theory now awaits the accumulation of additional data. At present it may be said that rare crossovers are often of different kinds in different families and that they emerge very unevenly within families.

SUMMARY

Certain results of oögonial crossing over should be detectable in spite of their partial masking by the large amount of meiotic crossing over in Drosophila females. In individual families and subfamilies a single oögonial crossing over may be inferred from (1) an excess of total recombination in any one region, (2) complementary crossover classes which are unequal after allowing for viability differences, (3) decreased recombination in one or all regions distal to a region of increase.

In the combined data of whole experiments, indications of many exchanges in various gonial cells during early or late mitoses could be (4) increases in recombination above expected values which are greatest at the spindle attachment and progressively less, or even negative, in distal regions, (5) altered interference values and, finally, (6) non-random distribution of rare crossover offspring. Each of the above expectations, derived chiefly from figure 1, has already been reported independently as an observed fact. No single experiment is known to the author which shows all of the above indications of gonial crossing over in unmistakable relationship, but a promising one has been planned. Since good examples may occur anywhere in a wide variety of experiments, the writer would appreciate it if other research workers would be on the look-out for such signs of gonial crossing over as have been discussed above and for any others which may come to mind.

LITERATURE CITED

BEADLE, G. W., and S. EMERSON, 1935 Further studies on crossing-over in attached-X chromosomes of *Drosophila melanogaster*. Genetics **20**: 192–206.

COOPER, KENNETH W., 1949 The cytogenetics of meiosis in Drosophila. Mitotic and meiotic autosomal chiasmata without crossing-over in the male. J. Morph. **84**: 81–122.

FRIESEN, H., 1936 Spermatogoniales Crossing Over bei Drosophila. Z. I. A. V. **71**: 501–526.

KIKKAWA, HIDEO, 1934 Studies on non-inherited variation in crossing over in Drosophila. J. Genet. **28**: 329–348.

MAVOR, J. A., 1923 An effect of X-rays on the linkage of Mendelian characters in the first chromosome of Drosophila. Genetics **8**: 355–366.

SCHULTZ, J., and H. REDFIELD, 1932 (in Morgan, Bridges and Schultz) Carnegie Instn. Wash. Yearb. **31**: 306.

1933 (in Morgan, Bridges and Schultz) Carnegie Instn. Wash. Yearb. **32**: 299.

SEREBROVSKY, A. S., 1927 The influence of the "purple" gene on the crossing over between "black" and "cinnabar" in *Drosophila melanogaster*. J. Genet. **18**: 137–175.

STEINBERG, A. C., and F. CLARKE FRASER, 1944 Studies on the effect of X-chromosome inversions on crossing over in the third chromosome of *Drosophila melanogaster*. Genetics **29**: 83–103.

STERN, C., 1936 Somatic crossing over and segregation in *Drosophila melanogaster*. Genetics **21**: 625–730.

WHITTINGHILL, M., 1937 Induced crossing over in Drosophila males and its probable nature. Genetics **22**: 114–129.

1938 Oögonial crossing over in *Drosophila melanogaster*. Genetics **23**: 175–176.

1948a A spermatogonial map of chromosome 3 of *Drosophila melanogaster*. Genetics **33**: 131;

1948b The effects of methyl-bis (b-chloroethyl)amine upon recombination values in *Drosophila melanogaster*. Genetics **33**: 634.

# THE INFLUENCE OF HIGH AND LOW TEMPERATURES ON THE EXPRESSION OF TUMOROUS HEAD IN DROSOPHILA MELANOGASTER

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Received May 18, 1949

IN a previous paper (GARDNER and WOOLF 1949) a character expressed as abnormal growths in the head of *Drosophila melanogaster* was discussed. The expression was shown to be dependent upon two genes, one sex-linked and one autosomal. The sex-linked gene (*tu-1*) was shown to control a maternal effect expressed only in the presence of the third chromosome semidominant gene *tu-3*). The penetrance in the inbred stock (symbolized *tu-h*) homozygous for *tu-1* and *tu-3*, was 76 percent at 22°C. Outcrosses involving females from the *tu-h* stock and males from laboratory stocks produced an average of about 30 percent of abnormal flies in the $F_1$ at 22°C. Reciprocal outcrosses between *tu-h* males and females from laboratory stocks other than Oregon produced less than 1 percent of abnormal flies in the $F_1$. Oregon females were shown to behave like *tu-h* females in producing the maternal effect. Small percentages, not exceeding five percent of abnormal flies were obtained in the $F_2$ from both reciprocal crosses involving all except one of the laboratory stocks studied. Higher percentages were obtained from crosses involving a stock containing aristaless, dumpy, dachs, black, curved, plexus and speck on one second chromosome and Curly and speck on the other second chromosome (*al dp d b c px sp/Cy sp*) suggesting the presence of a modifier in this stock.

The objective of the present paper is to report the results of experiments designed to determine the effect of different temperatures upon the expression of the character. Preliminary investigations indicated that the penetrance and expressivity increase proportionally with temperatures from 15°C to 30°C. The viability was shown to be considerably decreased at 30°C, the females showing a greater inviability than the males at temperatures above 22°C. The preliminary results also indicated a temperature effective period at an early stage in development. The flies were raised in half-pint milk bottles on the standard cornmeal, agar, molasses media.

## METHODS AND EXPERIMENTAL DATA

### *Effects of Different Temperatures*

Matings were prepared from the inbred *tu-h* stock and the cultures were subjected to temperatures of 15°, 18°, 21°, 25°, 28°, and 30° C during the early stages of development of the progeny. The eggs were laid at the temperature indicated, the parent flies were removed and the cultures were allowed to remain at the same temperature for 24 hours. The results are summarized

in table 1. A progressive increase in penetrance was observed with increase in temperature between 18°C and 30°C.

Previous experiments (GARDNER and WOOLF 1949) indicated a genetic pattern involving a maternal effect in the presence of a third chromosome semidominant gene (*tu-3*). A small percentage (less than 1 percent) of abnormal flies was obtained from a number of crosses in the absence of the maternal effect. This small expression was attributed to the independent action of *tu-3*. Experiments were designed to verify this assumption and determine the influence of temperature upon the individual genes involved in the expression

TABLE 1

*Effect of different temperatures during the first 24 hours of development upon the expression in the inbred tumorous head stock.*

| TEMPERATURE | PROGENY | | | | |
|---|---|---|---|---|---|
| DEGREES C | ABN. ♀ | + ♀ | ABN. ♂ | + ♂ | AVERAGE % ABN. |
| 30±.5 | 177 | 5 | 559 | 48 | 93 |
| 28±.5 | 246 | 16 | 552 | 70 | 90 |
| 25±.5 | 756 | 102 | 776 | 280 | 80 |
| 21±1 | 632 | 258 | 595 | 304 | 68 |
| Room temperature | | | | | |
| 21±2 | 312 | 121 | 325 | 214 | 66 |
| 18±1 | 483 | 262 | 329 | 430 | 54 |
| 15±1 | 347 | 146 | 267 | 333 | 56 |

of the trait. By the use of markers a stock was prepared in which *tu-3* was eliminated from the *tu-h* stock and *tu-1* was retained in homozygous condition in the females. Crosses involving parents from this stock were subjected to 30°C for 24 hours. A total of 2164 flies was produced but no abnormal flies were found.

A stock was next prepared with *tu-1* eliminated and *tu-3* present in homozygous condition. Cultures from this stock were also subjected to temperature treatment. From a total of 1112 flies three were abnormal. This result is comparable with that of the previous study conducted at normal temperature and demonstrates the fact that increased temperature has no influence upon the expression of the abnormality in the absence of *tu-1*. Cultures involving females heterozygous for *tu-1* and homozygous for *tu-3* and males lacking *tu-1* but homozygous for *tu-3* were next subjected to the increased temperature. From a total of 329 progeny 2.4 percent were abnormal. This result is also comparable with that of the previous study conducted at normal temperature and demonstrates the fact that temperature treatment does not influence the expression of the character unless *tu-1* is homozygous. It might therefore be expected that a change in the maternal effect follows temperature treatment.

Reciprocal outcrosses with laboratory stocks were next prepared and the cultures were subjected to temperature treatment at 30°C during the first 24

TABLE 2

*Average percentage of abnormal flies in $F_1$ and $F_2$ from reciprocal outcrosses between tu-h and laboratory stocks at 30°C during first 24 hours of development. Controls were maintained at 22°C.*

| LABORATORY STOCK | $F_1$ | | | | | | | | $F_2$ | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | *tu-h*×LAB. STOCK | | | | RECIPROCAL CROSS | | | | *tu-h*×LAB. STOCK | | | | RECIPROCAL CROSS | | | |
| | 30° | | 22° | | 30° | | 22° | | 30° | | 22° | | 30° | | 22° | |
| | N | % | N | % | N | % | N | % | N | % | N | % | N | % | N | % |
| Oregon R | 195 | 89 | 817 | 38 | 267 | 81 | 986 | 42 | 64 | 83 | 621 | 20 | 267 | 72 | 1728 | 26 |
| Canton | 104 | 92 | 750 | 25 | 185 | 0 | 1542 | 0 | 203 | 0 | 540 | 0 | 79 | 0 | 329 | 0 |
| Florida | 94 | 92 | 299 | 39 | 157 | 0.6 | 220 | 1 | 234 | 14 | 96 | 4 | 173 | 13 | 287 | 1 |
| Lausanne | 48 | 85 | 872 | 29 | 182 | 1.6 | 200 | 0 | 620 | 0.6 | 1142 | 0.6 | 253 | 0 | 669 | 0.9 |
| Muller I[13] | 156 | 89 | 361 | 37 | 935 | 0 | 289 | 0 | 468 | 1 | 531 | 1 | 496 | 1.2 | 537 | 1 |
| *$Bd^g$/In 3R Cl3a* | 77 | 86 | 157 | 20 | 101 | 0 | 53 | 0 | 462 | 6.3 | 328 | 2 | 1761 | 23 | 473 | 4 |
| *ClB; Cy/Pm; H/Sh* | 185 | 87 | 90 | 39 | 1294 | 0.2 | 88 | 1 | 374 | 0.3 | 580 | 1 | 1239 | 1.6 | 320 | 1 |
| *D/Gl* | 119 | 66 | 460 | 15 | 557 | 1.3 | 132 | 0 | 969 | 2.1 | 956 | 4 | 425 | 0.9 | 331 | 0.6 |
| *$H^2$/Xa* | 193 | 76 | 361 | 23 | 375 | 0.3 | 174 | 0 | 1812 | 0.3 | 78 | 0 | 593 | 0.7 | 472 | 0.2 |

hours of development. Table 2 summarizes the $F_1$ and $F_2$ results of these crosses and also includes the results of parallel control crosses at normal temperature. The $F_1$ results between *tu-h* females and males from laboratory stocks showed a marked increase in percentage of abnormal flies following heat treatment. The percentage of abnormal flies from the reciprocal crosses, except the one with Oregon, were low, and compared favorably with the controls. Crosses involving Oregon produced comparable results for both reciprocal crosses. Oregon was shown in the previous study (GARDNER and WOOLF 1949) to carry a maternal effect like *tu-h*. This increase in effectiveness at high temperature, parallel with that of *tu-h*, supports the previous conclusion that Oregon and *tu-h* carry the same sex-linked factor which controls the maternal effect.

The expression in the $F_2$ was low and the results from the treated flies in general were comparable with those of the controls. Oregon again was an exception and followed the pattern expected on the basis of the previous study. Two other stocks, *Bd$^g$/In 3R Cl3a* and Florida, produced a greater expression than most others at normal temperature. The results involving these stocks showed a slight increase in percentage of abnormality above that at normal temperature, following temperature treatment. This suggests the presence of modifiers which may be influenced to a limited extent by temperature during the first 24 hours of development of the flies.

### *Temperature Effective Period*

#### *Cold Treatments*

Evidence for a temperature effective period during the first 24 hours of development was obtained from investigations made at high and low temperatures. The effects of cold treatment at 15°C were determined from studies involving four groups of abnormal parent flies from the inbred *tu-h* stock. There were approximately 20 flies in each group. The stocks from which the flies were taken were showing consistently an average penetrance of 75 percent at 21°C. The groups of parent flies were transferred to fresh culture bottles every 24 hours and eggs were deposited in the bottles. The bottles containing the developing flies were maintained at 21°C except during the period of cold treatment. The cultures were placed in the 15°C incubator beginning at 0,1, 2, 3, 4, 5, or 6 days after the eggs were laid and allowed to remain at 15°C for 2, 4, 6, or 8 to 10 days. A total of 28 cultures was given the cold treatment for varying lengths of time, during different developmental periods. The design of the experiment is indicated in table 3. The four control cultures were maintained at 21°C throughout the experiment. The four cultures of series 1 were in the 15°C incubator at the time the eggs were laid and the individual cultures remained at the cold temperature for 2, 4, 6, and 8 days. The eggs for series 2 were laid at 21°C and the cultures were placed in the 15°C incubator at the end of the first 24 hours. These cultures remained at the low temperature until the 3rd, 5th, 7th, and 10th day of development. Other cultures were treated as indicated in the table. A total of 5287 adult flies resulting from the treated cultures was classified.

Table 3 summarizes the results from the treated cultures and controls.

TABLE 3

*Effect of 15°C treatment on the expression of the tumorous head inbred stock at different developmental periods.*

| SERIES | DAYS OF DEVELOPMENT | | PROGENY | | | | |
|---|---|---|---|---|---|---|---|
| | TREATMENT BEGUN | ENDED | TOTAL ♀ | % ABN. ♀ | TOTAL ♂ | % ABN. ♂ | AVG. % ABN. |
| Control | — | — | 54 | 83 | 75 | 53 | 66 |
| | — | — | 76 | 92 | 92 | 73 | 82 |
| | — | — | 73 | 88 | 91 | 77 | 82 |
| | — | — | 64 | 95 | 61 | 77 | 86 |
| 1 | 0 | 2 | 70 | 61 | 81 | 23 | 41 |
| | 0 | 4 | 20 | 60 | 19 | 47 | 54 |
| | 0 | 6 | 25 | 80 | 26 | 27 | 53 |
| | 0 | 8 | 25 | 72 | 37 | 62 | 66 |
| 2 | 1 | 3 | 158 | 82 | 162 | 65 | 73 |
| | 1 | 5 | 112 | 87 | 89 | 64 | 77 |
| | 1 | 7 | 96 | 91 | 92 | 66 | 79 |
| | 1 | 10 | 93 | 87 | 88 | 76 | 82 |
| 3 | 2 | 4 | 156 | 85 | 170 | 64 | 76 |
| | 2 | 6 | 56 | 91 | 81 | 69 | 78 |
| | 2 | 8 | 77 | 84 | 77 | 67 | 75 |
| | 2 | 12 | 68 | 87 | 75 | 68 | 77 |
| 4 | 3 | 5 | 165 | 89 | 168 | 64 | 76 |
| | 3 | 7 | 77 | 82 | 114 | 55 | 66 |
| | 3 | 9 | 75 | 83 | 50 | 62 | 74 |
| | 3 | 13 | 100 | 87 | 99 | 67 | 77 |
| 5 | 4 | 6 | 74 | 95 | 117 | 74 | 82 |
| | 4 | 8 | 75 | 79 | 89 | 63 | 70 |
| | 4 | 10 | 69 | 80 | 72 | 65 | 72 |
| | 4 | 13 | 117 | 90 | 115 | 79 | 84 |
| 6 | 5 | 7 | 53 | 87 | 77 | 74 | 79 |
| | 5 | 9 | 106 | 76 | 141 | 58 | 66 |
| | 5 | 11 | 99 | 85 | 100 | 63 | 74 |
| | 5 | 14 | 101 | 85 | 116 | 69 | 76 |
| 7 | 6 | 8 | 40 | 77 | 28 | 57 | 69 |
| | 6 | 10 | 50 | 78 | 72 | 57 | 66 |
| | 6 | 12 | 57 | 84 | 65 | 55 | 69 |
| | 6 | 15 | 29 | 93 | 38 | 66 | 78 |

No significant deviation from the controls was observed from any of the cultures except the first group (series 1) which included the flies treated at the earliest period. The average penetrance in these cultures was significantly lower than that of the controls and all other groups. This suggests a critical period within the first 48 hours of development under the conditions of the experiment. The penetrance in the controls ranged from 66 percent to 86 percent and all treated cultures except those in series 1 fell within the same range. The penetrance in series 1 varied from 41 percent to 66 percent. In this series the eggs were laid at 15°C and the period of temperature treatment included the early developmental period. The culture treated during the first

two days of development gave interesting results. In a total of 151 flies, only 41 percent expressed the abnormality. This penetrance is considerably below that of the controls and all other cultures treated at later developmental periods. The other three cultures in series 1 were given longer temperature treatment and produced smaller numbers of progeny than any other cultures in the entire experiment. The culture given treatment for four days produced 39 flies with a penetrance of 54 percent, the one treated six days had 51 flies with a penetrance of 53 percent and the one given eight days treatment produced 62 flies, of which 66 percent were abnormal. These results suggest a viability problem associated with the tumorous head abnormality. This problem will be discussed in another paper.

TABLE 4

*Percentage of abnormal flies in the $F_1$ from crosses between tu-h females and males from laboratory stocks at 15°C during early critical period.*

| LABORATORY STOCK | TOTAL FLIES | % ABNORMAL |
|---|---|---|
| Oregon | 372 | 20 |
| Canton | 217 | 17 |
| Florida | 115 | 19 |
| Lausanne | 202 | 14 |
| Urbana | 144 | 22 |
| *$w^a$* | 92 | 13 |
| *al dp d b c px sp/Cy sp* | 251 | 8 |
| *$Bd^Q$/In 3R Cl3a* | 278 | 22 |
| *D/Gl* | 88 | 14 |
| *$H^2$/Xa* | 214 | 22 |
| *Cy/Pm; H/Sb* | 32 | 16 |

A higher penetrance was observed among the females than among the males in all groups including the controls. The average percent of abnormal females produced in the controls varied from 83 percent to 95 percent and the average percent for abnormal males ranged from 53 percent to 77 percent, showing a significant difference. When the cold treatment was applied during the first 48 hours, 61 percent of the females were abnormal compared with 23 percent of abnormal males. The expressivity of the treated flies and the location of the growths on the head were not significantly different from the controls.

The next experiment was designed to determine the effect of low temperature during the early developmental period on the results of outcrosses between *tu-h* females and males from laboratory stocks. The parent flies were introduced into fresh, cool culture bottles and placed immediately in the 15°C incubator. The eggs were laid at 15°C. At the end of a 24 hour egg laying period the parents were removed and the cultures containing the eggs were allowed to remain for an additional 24 hours at 15°C. They were then transferred to 21°C and maintained at that temperature until the experiment was completed.

The results of the outcrosses are summarized in table 4. An average of about 17 percent of abnormal flies was obtained. This average percentage is significantly lower than the average of about 30 percent of abnormal flies obtained

from the results of similar outcrosses at normal temperature which were reported in a previous paper (GARDNER and WOOLF 1949). The results of these outcrosses subjected to low temperature treatment support the conclu-

TABLE 5

*Effect of 31°C treatment on expression of inbred tumorous head stock at different developmental periods.*

| SERIES | DAYS OF DEVELOPMENT | | PROGENY | | | | |
|---|---|---|---|---|---|---|---|
| | TREATMENT BEGUN | ENDED | TOTAL ♀ | % ABN. ♀ | TOTAL ♂ | % ABN. ♂ | AVG. % ABN. |
| Control | — | — | 43 | 79 | 44 | 50 | 64 |
| | — | — | 50 | 86 | 74 | 62 | 72 |
| | — | — | 28 | 86 | 40 | 75 | 79 |
| | — | — | 28 | 93 | 41 | 83 | 87 |
| 1 | 0 | 2 | 5 | 100 | 94 | 86 | 87 |
| | 0 | 4 | 6 | 100 | 79 | 78 | 80 |
| | 0 | 6 | 1 | 100 | 26 | 77 | 78 |
| | 0 | 8 | 5 | 100 | 33 | 82 | 84 |
| 2 | 1 | 3 | 58 | 81 | 79 | 71 | 75 |
| | 1 | 5 | 30 | 80 | 55 | 65 | 71 |
| | 1 | 7 | 51 | 96 | 49 | 86 | 91 |
| | 1 | 9 | 29 | 93 | 34 | 85 | 89 |
| 3 | 2 | 4 | 74 | 88 | 80 | 70 | 79 |
| | 2 | 6 | 80 | 86 | 98 | 54 | 71 |
| | 2 | 8 | 34 | 85 | 56 | 54 | 66 |
| | 2 | 10 | 50 | 76 | 52 | 52 | 64 |
| 4 | 3 | 5 | 63 | 90 | 80 | 56 | 71 |
| | 3 | 7 | 53 | 92 | 60 | 62 | 76 |
| | 3 | 9 | 65 | 91 | 54 | 72 | 83 |
| | 3 | 11 | 33 | 94 | 48 | 87 | 90 |
| 5 | 4 | 6 | 21 | 90 | 65 | 58 | 66 |
| | 4 | 8 | 32 | 87 | 60 | 65 | 73 |
| | 4 | 10 | 55 | 98 | 71 | 83 | 90 |
| | 4 | 12 | 30 | 93 | 43 | 67 | 78 |
| 6 | 5 | 7 | 16 | 81 | 63 | 59 | 63 |
| | 5 | 9 | 10 | 100 | 77 | 70 | 74 |
| | 5 | 11 | 44 | 98 | 68 | 78 | 86 |
| | 5 | 13 | 48 | 92 | 53 | 75 | 83 |
| 7 | 6 | 8 | 23 | 83 | 22 | 64 | 73 |
| | 6 | 10 | 41 | 90 | 65 | 54 | 68 |
| | 6 | 12 | 65 | 91 | 83 | 61 | 74 |
| | 6 | 14 | 36 | 78 | 24 | 54 | 68 |

sion drawn from the previous low temperature experiments involving the inbred *tu-h* stock by indicating an early temperature effective period. Some comparable outcrosses were subjected to low temperature at other periods of development. No significant deviation from the controls was observed in the results of these crosses. This indicates that the only conspicuous temperature effective period occurs during the first 48 hours of development.

*Heat Treatments*

The first heat treatments were conducted in the same way as the cold treatments. The incubator was set at 31°C. The four groups of parent flies were taken from the same stocks as the parents used in the cold treatments. A total of 3,021 flies was classified. The results are summarized in table 5. No significant differences were observed in the averages of the groups of cultures in penetrance or expressivity. An analysis of the results, however, revealed some significant differences in viability between groups of cultures. All of the females from the four cultures subjected to 31°C during the first 48 hours (series 1) were abnormal but only a comparatively few females were produced. The sex ratio was strikingly unequal. The total for the four bottles was 232 males compared with 17 females. A slightly greater number of males than females was observed in results of the controls and most of the other groups of cultures. The tendency toward the greater numbers of males in the *tu-h* stock is exaggerated by the temperature treatment during the first 48 hours of development. Other studies (GARDNER, in press, WOOLF in press and unpublished data) have also indicated a greater penetrance, expressivity and inviability among the females than among the males from the *tu-h* stock at normal temperatures.

The lack of a significant average difference in penetrance following heat treatment during the early period is explained largely on the basis of viability. The heat is believed to have increased the expressivity to a degree which made it impossible for many of the abnormal flies to survive. More abnormal flies died during development than normal flies and only those abnormal flies with less extreme abnormalities survived. Since females express the abnormality more extremely than males, more of them were killed and a greater proportion of males survived. This explanation is supported by the viability studies which will be reported in another paper. The heat at this early period apparently exaggerated the tendency toward differential expression and survival which was observed at normal temperatures. When special precautions were taken to see that all eggs were allowed to remain 24 hours at 30°C an average penetrance of 93 percent was obtained. This is the highest percentage of abnormal flies obtained so far from a large sample of *tu-h* flies. Since results of outcrosses subjected to low temperature during the early developmental period had shown a marked decrease in percentage of abnormal flies, studies were next designed to determine the effect of high temperature on outcrosses during the same period. These experiments were conducted in the same way as the cold treatments and heat treatments described above, but the incubator was set at 30°C. Crosses were made between *tu-h* females and Lausanne males. Since experimental results had indicated a shorter temperature effective period, intervals of 24 hours were used for this study instead of 48 hours as in the previous experiments. The results are summarized in table 6. Significantly higher percentages of abnormal flies were obtained from the cultures subjected to 30°C during the first 24 hours of development (series 1) than from the controls.

An average of 87 percent of abnormal flies was obtained from the six cultures in series 1 compared with an average of 27 percent for the controls at

TABLE 6

*Effect of 30°C treatments on the expression of tumorous head in the $F_1$ progeny from crosses between tu-h females and Lausanne males at different developmental periods.*

| SERIES | DAYS OF DEVELOPMENT | | PROGENY | | | | |
|---|---|---|---|---|---|---|---|
| | TREATMENT BEGUN | ENDED | TOTAL ♀ | % ABN. ♀ | TOTAL ♂ | % ABN. ♂ | AVG. % ABN. |
| Control | — | — | 52 | 46 | 46 | 20 | 34 |
| | — | — | 82 | 33 | 89 | 18 | 25 |
| | — | — | 82 | 27 | 72 | 10 | 19 |
| | — | — | 63 | 24 | 54 | 20 | 22 |
| | — | — | 82 | 38 | 58 | 31 | 35 |
| 1 | 0 | 1 | 113 | 82 | 233 | 73 | 76 |
| | 0 | 2 | 109 | 83 | 123 | 75 | 79 |
| | 0 | 3 | 39 | 92 | 46 | 78 | 85 |
| | 0 | 4 | 13 | 92 | 28 | 86 | 88 |
| | 0 | 5 | 22 | 100 | 43 | 93 | 95 |
| | 0 | 6 | 4 | 100 | 20 | 95 | 96 |
| 2 | 1 | 2 | 224 | 37 | 237 | 35 | 36 |
| | 1 | 3 | 130 | 46 | 115 | 22 | 35 |
| | 1 | 4 | 22 | 45 | 20 | 30 | 38 |
| | 1 | 5 | 105 | 58 | 79 | 37 | 49 |
| | 1 | 6 | 5 | 20 | 4 | 25 | 22 |
| 3 | 2 | 3 | 233 | 36 | 218 | 27 | 32 |
| | 2 | 4 | 123 | 38 | 95 | 32 | 35 |
| | 2 | 5 | 122 | 35 | 87 | 34 | 35 |
| | 2 | 6 | 107 | 50 | 104 | 27 | 39 |
| | 2 | 7 | 46 | 35 | 64 | 19 | 25 |
| 4 | 3 | 4 | 238 | 53 | 180 | 33 | 44 |
| | 3 | 5 | 122 | 52 | 123 | 37 | 44 |
| | 3 | 6 | 149 | 48 | 111 | 39 | 44 |
| | 3 | 7 | 90 | 57 | 83 | 47 | 52 |
| | 3 | 8 | 93 | 37 | 96 | 35 | 36 |
| 5 | 4 | 5 | 296 | 45 | 299 | 34 | 39 |
| | 4 | 6 | 194 | 47 | 162 | 45 | 46 |
| | 4 | 7 | 167 | 55 | 175 | 40 | 47 |
| | 4 | 8 | 99 | 53 | 118 | 39 | 45 |
| | 4 | 9 | 43 | 51 | 62 | 24 | 35 |
| 6 | 5 | 6 | 155 | 28 | 136 | 19 | 24 |
| | 5 | 7 | 156 | 33 | 163 | 22 | 27 |
| | 5 | 8 | 176 | 47 | 161 | 24 | 36 |
| | 5 | 9 | 100 | 40 | 94 | 24 | 32 |
| 7 | 6 | 7 | 195 | 16 | 162 | 12 | 15 |
| | 6 | 8 | 182 | 26 | 156 | 13 | 20 |
| | 6 | 9 | 187 | 32 | 178 | 18 | 25 |
| | 6 | 10 | 29 | 24 | 46 | 22 | 23 |

normal temperature. The proportion of abnormal flies produced from the outcrosses subjected to 30°C during the temperature effective period was slightly higher than that of the inbred stock under the same treatment at 31°C and considerably higher than that of the outcrosses at normal temperature. The same number of *tu-1* genes was present in the mothers involved in all crosses and a comparable maternal effect might be expected. The *tu-3* gene was heterozygous in the progeny resulting from the outcrosses and homozygous in the flies from the inbred stock. The viability factor was evidently more effective in the inbred stock.

No striking differences were observed from cultures subjected to treatment at any other period. A slight increase in penetrance was obtained from cultures subjected to the higher temperatures during the third and fourth day of development. This may suggest a later, less conspicuous temperature effective period. From these studies it is concluded that the first 24 hours of development is a critical period during which changes in temperature may influence the expression of the tumorous head abnormality.

Further studies designed to determine whether or not a shorter critical period exists within the first 24 hours, have been conducted at four hour intervals. No significant increase in penetrance has been observed for shorter periods. Twenty-four hours appears to be the minimum effective period. The length of the period beyond 24 hours is not important with respect to penetrance and expressivity but viability is decreased when the heat is applied for longer duration.

Experiments designed to determine the effect of temperature pretreatment upon males and virgin females have also been conducted. The results showed no significant effect following pretreatment on either males or unfertilized females. Females, previously fertilized, produce eggs which show the same results when subjected to 30°C during the first 24-hour period of development as those produced by females treated in the presence of the males. This demonstrates further that the heat treatment on the male has no influence on the expression of the trait.

## DISCUSSION

The *tu-h* stock has been inbred and selected over a period of two years. Attempts have been made to add genetic modifiers and thus increase the expression but so far a 100 percent penetrance has not been obtained. The present study was designed to determine the effects of environmental temperature upon the penetrance and expressivity of the character. Temperature is known to effect the expression of many mutant characters (PLUNKETT 1926; GOLDSCHMIDT 1938; HARNLY 1930, 1932; HERSH and WARD, 1932; STANLEY 1925; CHILD 1936; IVES 1939; VILLEE 1942, 1943; and others). In most cases the increase in temperature is followed by a shift in the mutant phenotype toward the normal and can be explained on the basis of changes in developmental rates. In some cases, increased temperature increases the abnormality or deviation from the wild pattern. In the present study the expressivity was ap-

preciably increased at higher temperatures and the penetrance was increased to 93 percent at 30°C.

HARTUNG (1947) has studied the effects of temperatures from 20°C to 30°C on five tumor bearing strains of *Drosophila melanogaster*. Three strains showed highest incidence of tumors at the lowest temperature. One showed little change except for a decline at the highest temperature and one showed highest incidence between 23°C and 28°C with a marked decline at either end of the range. These data suggest that high temperatures inhibit tumors. Data presented in the present report show that increased temperature increases the incidence of head abnormalities. RUSSELL (1942) has described the inheritance of one of the tumor strains studied by HARTUNG and found the expression of tumors to be dependent upon (1) a second chromosome tumor factor, (2) a sex-linked factor which enhances the action of the tumor factor and (3) a third chromosome inhibitor. Previous investigations (GARDNER and WOOLF 1949) have shown that the expression of tumorous head is dependent upon two factors: (1) the basic factor in the third chromosome without which no head growths appear and (2) a sex-linked factor which produces a maternal effect increasing the percentage of abnormal $F_1$ flies from less than 1 percent to about 30 percent in outcrosses at 22°C. The present data have shown that the increased temperature enhances the maternal effect and thus increases the percentage of abnormal $F_1$ flies to about 85 percent at 30°C. Temperature has no influence upon the action of the third chromosome factor in the absence of the maternal effect.

Temperature effective periods for the expression of many mutant characters and phenocopies have been described by the authors cited above and others (VOGT 1946; CHILD, BLANC and PLOUGH 1940). Most of these periods at which the expression of a character can be changed by temperature have been identified during the latter part of the larval-developmental period. Some are very specific and some extend over a longer developmental period. The present data have demonstrated an early temperature effective period during the first 24 hours of development, in the egg stage, which is associated with a maternal effect. This early period must represent the stage of development when basic morphogenic substances are being elaborated in the egg. VOGT (1947) has described an early temperature effective period for the mutant *antennaless*. PLUNKETT (1926) has analyzed a less specific effect on bristle characters.

## SUMMARY

The expression of tumorous head is increased progressively when the flies from the inbred stock are subjected to increased temperatures from 18°C to 30°C. A maximum penetrance of 93 percent was obtained at 30°C. Two factors are involved in the expression of the character, a third chromosome basic factor and a sex-linked factor producing a maternal effect. The maternal effect is influenced by temperature change but the action of the third chromosome factor is not changed. A temperature effective period during the first 24 hours of development, in the egg and early larval stage, has been identified.

ACKNOWLEDGMENT

The writers acknowledge with thanks the assistance of DR. HORACE DAVENPORT, DR. W. W. NEWBY and DR. F. E. STEPHENS who have read the manuscript and made suggestions.

The project was supported by a grant from the UNIVERSITY OF UTAH RESEARCH FUND.

LITERATURE CITED

CHILD, G. P., 1936 III. The effect of temperature on scute 5. Genetics **21**: 808–816.

CHILD, G. P., R. BLANC, and H. H. PLOUGH, 1940 Somatic effects of temperature on development in *Drosophila melanogaster*. Physiol. Zool. **13**: 56–64.

GARDNER, ELDON J., In press. Head tumors in *Drosophila melanogaster*. Proc. Utah Acad. Sci. Arts and Letters. (Abstract).

In press. Inheritance of tumorous head in *Drosophila melanogaster*. Proc. Utah Acad. Sci. Arts and Letters. (Abstract).

GARDNER, ELDON J., and CHARLES M. WOOLF, 1949 Maternal effect involved in the inheritance of abnormal growths in the head region of *Drosophila melanogaster*. Genetics. **34**: 573–585.

GOLDSCHMIDT, R., 1938 Physiological Genetics. ix+375 pp. McGraw-Hill Book Co. N. Y.

HARNLY, MORRIS, H., 1930 A critical temperature for lengthening of the vestigial wings of *D. melanogaster* with sexually dimorphic effects. J. Exper. Zool. **56**: 363–368.

1932 The temperature effective period for the lengthening of the vestigial wings in Drosophila. Proc. Sixth Internat. Cong. Genetics **2**: 79–81.

HARTUNG, E. W., 1947 Some effects of temperature on tumor incidence in several strains of *Drosophila melanogaster*. J. Exper. Zool. **106**: 223–232.

HERSH, A. H., and ESTHER WARD, 1932 The effect of temperature on wing size in reciprocal heterozygotes of vestigial in *Drosophila melanogaster*. J. Exper. Zool. **61**: 223–244.

IVES, P. T., 1939 The effect of high temperature on bristle frequencies in scute and wild type males of *Drosophila melanogaster*. Genetics **24**: 315–331.

PLUNKETT, C. R., 1926 The interaction of genetic and environmental factors in development. J. Exper. Zool. **46**: 180–244.

RUSSELL, E. S., 1942 The inheritance of tumors in *Drosophila melanogaster* with an especial reference to an isogenic strain of st sr tumor 36a. Genetics **27**: 612–618.

STANLEY, W. F., 1935 The effect of temperature upon wing size in Drosophila. J. exp. Zool. **69**: 459–495.

VILLEE, C. A., 1942 The effect of cold treatments upon the development of the mutant aristapedia-Bridges in *Drosophila melanogsater*. Genetics **27**: 73.

1943 Phenogenetic studies of aristapedia alleles of *Drosophila melanogaster*. Genetics **28**: 94.

VOGT, M., 1946 Zur labilen Determination der Imaginalscheiben von Drosophila 1. Verhalten verschiedenaltriger Imaginalanlagen bei operativer Defektsetzung. Biol. Zbl. **65**: 223–238.

1947 Zur labilen Determination der Imaginalscheiben von Drosophila. V. Beitrag zur Manifestierung der Mutante antennaless. Biol. Zbl. **66**: 388–395.

WOOLF, CHARLES M., In press. Temperature effects on head tumors in *Drosophila melanogaster*. Proc. Utah Acad. Sci. Arts and Letters. (Abstract).

# ON THE INTERPRETATION OF THE DOSE-FREQUENCY CURVE IN RADIOGENETICS

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Received May 31, 1949

THE experiments of E. CASPARI and C. STERN (1948) on mutation of Drosophila at very low radiation intensities have put some doubt as to the reliability of the single event theory in the induction of mutations by ionizing radiations. Although the more recent experiments by D. E. UPHOFF and C. STERN (1949) imply a modification of the conclusions of that earlier paper, a critical analysis of the way through which the single event theory has been obtained in radiogenetics seems to be worthwhile.

The linearity of the relationship between the dose of an ionizing radiation and the frequency of induced mutations has been considered as a strong indication, if not as a proof, that mutation is induced by one primary event, which is usually interpreted as an ionization in a gene. This argument can be presented in the following manner:

It has been shown by physicists in a convincing manner that distribution of ionizations in the space is random. Consequently if we consider a cubic micron of a sample and a particular gene in it, the probability of occurrence of $n$ ionizations within that gene when $N$ ionizations are produced in that cubic micron is:

$$\binom{N}{n} p^n (1-p)^{N-n} \tag{1}$$

where $p$ is the probability that an ionization occurs in the gene if it occurs in the cubic micron of the sample. Since one roentgen produces about 2 ionizations per cubic micron and since it takes thousands of roentgen to produce few percent of mutations it may be conjectured that $p$ is a small number, although if $p$ were large but if it took many ionizations in the gene to produce a detectable mutation the frequency of the latter per roentgen would be still small. However other more direct and quite convincing arguments for the smallness of the gene (MULLER 1941) make it safe to consider the probability $p$ as a very small number. But if $p$ is small with respect to unity the expression (1) can be written as

$$\binom{N}{n} p^n. \tag{2}$$

Since $N$ is proportional to the dose, the only possibility compatible with the proportionality of (2) to the dose is $n=1$. In this way the single event theory could be considered as derived from the frequency to dose proportionality.

However to what extent is this linearity significant in view of the scattering of the experimental data? The mutation frequencies are known only within a small part of their statistical range and it is fair to ask whether the apparent linearity of the frequency-dose curve may be due to our limited knowledge about it. To answer this question it is here shown that assumptions *opposite* to those underlying the single event theory lead to a relationship which fits the experimental data equally well as the single event theory. *It is not the intention of this note to prove or disprove this or other theories in radiogenetics, but to point*

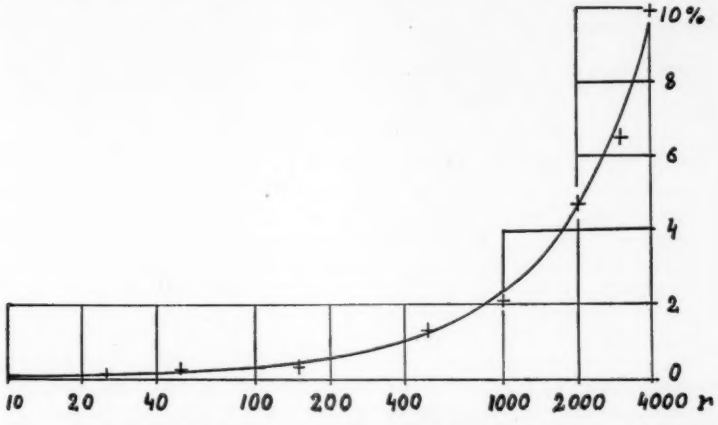


FIGURE 1.—Frequency of lethal mutations in Drosophila against dose in roentgen. The crosses are experimental data (SPENCER and STERN 1948). The curve is calculated from equations (3) and (4) with $S=42$ roentgen, $M=6600$ roentgen, $h=0.000025$ (roentgen)$^{-1}$. The relatively low value of $S$ expresses the low frequency of spontaneous mutations if compared with the induced mutations.

A linear fit of the experimental data by the least squares method gives:

$$P(D)=0.0000234788D+0.0001852.$$

There is no graphically expressible difference between this equation and the curve of the figure.

*out only that the apparent linearity of the frequency-dose curve should not be considered as a proof of the single event theory.*

Let us assume for a moment that not one but that many primary ionizing events must occur to produce a mutation. Consider a particular gene in the irradiated sample. Let one event be induced in that gene by a dose $\Delta D$. Since this event either consists of or is due to an ionization and since the occurrence of an ionization at a particular point in the space is a random event, $\Delta D$ is a random variable in so far as the induction of that event in the gene is concerned. If the induction of a detectable mutation requires many of such events in the gene and if all these events have a primary character, that is if they are independent of each other, the dose $D$ inducing such a mutation in the gene is a sum of many independent random variables like $\Delta D$. It is a fundamental theorem of the theory of probability, established under very general conditions, that a sum of independent random variables tends to the normal law of proba-

bility as the number of the variables tends to infinity (see *e.g.* CRAMER 1946, p. 215). Consequently the probability that a detectable mutation be induced in the gene with a dose between $A$ and $B$ is, with a good approximation if the number of primary events needed for the mutation is very large:

$$P = C \int_A^B \exp\,[-h^2(x - M)^2]dx$$

where $C$, $h$ and $M$ are constant. $B = D$ if this formula is applied to the usual type of experimental data on the dependence of the frequency of detected mutations upon the administered dose $D$. As to the lower limit of integration $A$ it cannot be taken equal to zero: because of the assumption made that the number of events necessary for the mutation is very large, the sample is to be considered as containing, at the time when the experiment starts, some effects identical or at least genetically equivalent to those which the radiation will induce in it. As a matter of fact it contains such effects, otherwise no spontaneous mutations would be detected in controls. Therefore, if we call $S$ the dose that would have to be given to the sample in order to produce in it these naturally existing effects, if it were free from them, then the frequency of mutations detected with a dose $D$ is:

$$P(D) = C \int_{-S}^{D} \exp\,[-h^2(x - M)^2]dx. \tag{3}$$

The condition $P(\infty) = 1$ determines the constant $C$. The frequency of spontaneous mutations is represented also by the above formula if $D = 0$ is put in it. $S$ is in a way a measure of the mutational status of the population from which the sample is taken. $P(D)$ appears in this way as a normal cumulative distribution truncated at $-S$. In numerical work with this formula it is convenient to put it in the following form:

$$1 - P(D) = [1 - H(hD - hM)]/[1 + H(hS + hM)] \tag{4}$$

where $H(x)$ is the function

$$(2/\sqrt{\pi}) \int_0^x \exp\,(-x^2)dx$$

tabulated by the WPA project for the CITY OF NEW YORK (1941). This formula for $P(D)$ has been fitted to W. P. SPENCER and C. STERN's data on X-ray induced lethal mutations in the X chromosome of *Drosophila melanogaster* (1948). Figure 1 shows this fit together with a straight line fit, the latter obtained by the least squares method. There is no significant difference between the two fits. The reason is that the function (3) is practically linear within the range of $D$ considered.

## SUMMARY

It is shown that assumptions opposite to those which are made in the single event theory of induction of mutations by radiation lead to a relationship

which is practically linear within its experimentally pertinent range. It is concluded from this that the apparent linearity of the experimental data on the dependence of the mutation frequency upon the administered dose cannot be considered as a proof that the mutation is caused by a single primary event.

## LITERATURE CITED

CASPARI, E., and C. STERN, 1948 The influence of chronic irradiation with gamma-rays at low dosages on the mutation rate in *Drosophila melanogaster*. Genetics **33**: 75–95.

CRAMER, H., 1946 Mathematical methods of statistics. xvi+575 pp. Princeton: University Press.

MULLER, H. J., 1941 Induced mutations in Drosophila. Cold Spring Harbor Symp. Quant. Biol. **9**: 151–167.

SPENCER, W. P., and C. STERN, 1948 Experiments to test the validity of the linear r-dose/mutation frequency relation in Drosophila at low dosage. Genetics **33**: 43–74.

UPHOFF, D. E., and C. STERN, 1949 The genetic effects of low intensity irradiation. Science **109**: 609–610.

WPA FOR THE CITY OF NEW YORK, 1941 Tables of Probability Functions. Vol. 1. xxviii+302 pp.

# THE EFFECTS OF GENETIC CHANGES ON TYROSINASE ACTIVITY IN GLOMERELLA

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Received June 6, 1949

ONE of the most significant and interesting problems in biology concerns the relationship between genes and enzymes. Recently considerable attention has been directed towards the hypothesis of genic action which states that the primary function of a gene lies in the control of enzyme synthesis (HOROWITZ 1949; BEADLE 1945, 1948; HALDANE 1942; WRIGHT 1941). In support of this concept several investigators have established correlations between the activity of an enzyme and the genetic constitution of an organism. However, the particular kind of correlation established varies with the different investigations. For example, MITCHELL and LEIN (1948) have shown that a single gene change completely prevents any measurable activity of the enzyme which catalyzes the coupling of serine and indole to form tryptophane in Neurospora. Similarly, SAWIN and GLICK (1943) working with atropinesterase from the blood serum of rabbits showed that this enzyme was inherited as an incompletely dominant trait. Homozygous recessive rabbits produced serum with no atropinesterase activity while the serum from the heterozygotes had about half the enzymatic activity of the serum from the dominant homozygotes. Thus, in this case, a single gene seems to exert an effect on the quantity of an enzyme produced.

On the other hand, the work of WINGE and ROBERTS (1948) on maltose fermentation by *Saccharomyces cerevisiae* indicates a polygenic control of enzyme synthesis. They found that any one of three non-allelic genes enabled *S. cerevisiae* to ferment maltose, presumably by bringing about the synthesis of maltase. A somewhat different kind of polygenic control of an enzyme, involving the degree of enzyme activity, is shown by the investigations of RUSSELL and RUSSELL (1948) on the intensity of dopa oxidase activity in the hair bulbs of the skin of mice. The dopa oxidase activity was found to be proportional to the intensity of yellow pigment in the hair and to be under the control of several different genes.

These examples of the genetic control of enzyme synthesis all involve measurements of the degree of enzymatic activity. Such differences in enzymatic activity may be attributed either to differences in the amount of the enzyme present or to different efficiencies of the same quantity of enzyme. Different efficiencies may in turn be explained by associated changes of the chemical milieu (pH changes, for example) in which the enzyme is acting or to inherent differences in the enzyme molecule itself. It is this last interpretation which forms the basis of the hypothesis (cf. BEADLE 1945) that the gene determines the specificity of the enzyme. Some of the support for this hypothesis stems

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from the demonstrated qualitative relationship between genes and specific protein antigens in the different blood groups. A similar kind of relationship may exist between genes and the protein enzymes. In fact, one investigator (SCHUURMAN 1937) has concluded that a qualitatively different enzyme is produced in the mealworm, *Tenebrio molitor*, as the result of a change in a single gene. Extracts prepared from the genetically different adult mealworms catalyzed the production of differently colored pigments from the same chromogen. However, since neither the chemical composition of the pigments nor the chemical steps in their synthesis are known, the enzymatic differences observed in the mealworm cannot be considered a conclusive verification of the one gene-one specific enzyme hypothesis.

These varied investigations on gene-enzyme relationships, taken together, seem to suggest that genes may control both the specific properties of an enzyme and also the quantity produced. In an effort to gather more information about gene-enzyme relationships, the present investigation was directed towards correlating changes in genetic constitution with the amount of tyrosinase activity in the fungus Glomerella.

## MATERIALS AND METHODS

### *Characteristics of Tyrosinase*

Several investigators have shown that the enzyme tyrosinase possesses properties which make it particularly useful for investigations of the genetic control of enzyme synthesis. Perhaps the most distinctive characteristic of tyrosinase is its ability to catalyze two quite different oxidations—that is, 1) the insertion of an hydroxyl group into monohydric phenols ortho to the one already present, and 2) the oxidation of many *o*-dihydric phenols to their corresponding *o*-quinones. Although some investigators doubt that tyrosinase is a single enzyme, NELSON and DAWSON (1944) and their collaborators have, over a period of several years, accumulated a large body of convincing evidence to show that tyrosinase is a single protein entity.

The relative rates of the two catalytic activities of tyrosinase extracted from the common mushroom *Psalliota campestris* are variable and depend upon such factors as the physical nature of the tyrosinase complex and the presence of other proteins. For example, the addition of gelatin to purified preparations of the enzyme alters the ratio of activities on the two kinds of substrates. Moreover, different methods of purification apparently change the physical nature of the tyrosinase, because, as NELSON and DAWSON (1944) have found, purified preparations with different ratios of activities also exhibit differences in their kinetic, substrate, inactivation, stability, and electrophoretic characteristics. These differences suggest that the different kinds of tyrosinase differ in the size or surface character of the enzyme molecule. It seems reasonable to suppose that such altered surface properties might also be induced by genetic changes which affect the synthesis of the enzyme complex. In fact, purified tyrosinase from the wild mushroom *Lactarius piperatus* is not identical with samples obtained from Psalliota. DALTON and NELSON (1939) were unable to

alter the ratio of catalytic activities of tyrosinase from Lactarius even by the same methods which were successful in the case of preparations from Psalliota. These results indicate that different kinds of tyrosinase may be produced by organisms of different genetic constitutions.

An additional reason for using tyrosinase in genetic studies stems from the extensive data on the inheritance of melanin pigmentation in mammals and birds. The initial steps in the formation of these pigments are apparently under the control of tyrosinase or dopa oxidase—an enzyme which is similar to, if not identical with, tyrosinase. Moreover, a large number of mutant genes have been found which affect the quantity and quality of the melanin pigments (WRIGHT 1942). These genetically controlled changes in pigmentation can also be correlated in some instances with changes in dopa oxidase activity (RUSSELL and RUSSELL 1948; GINSBURG and KAPLAN 1947; GINSBURG 1944). Thus the activity of tyrosinase seems to be particularly susceptible to genetic changes.

### *Tyrosinase in Glomerella*

Several different plants and animals are known to produce tyrosinase (KASTLE 1910; NELSON and DAWSON 1944). However, few of these organisms have characteristics which make them convenient tools for research in genetics. In an effort to find a suitable fungus for this investigation many black colored fungi were picked up at random and tested for their ability to produce tyrosinase, the assumption being that black pigment might indicate the presence of tyrosinase. Eventually a black-colored fungus, rich in tyrosinase, was found as a contaminant on an agar plate. This fungus was identified as a strain of the ascomycete *Glomerella cingulata* (Stoneman) Spauld. and v. Schr. by DR. DIEHL of the UNITED STATES DEPARTMENT OF AGRICULTURE. Confirmation of this identification was obtained by crossing the fungus with EDGERTON'S plus B strain of Glomerella, which was kindly supplied by S. J. P. CHILTON of LOUISIANA STATE UNIVERSITY. EDGERTON, CHILTON, LUCAS, and WHEELER during the last few years (1944, 1945, 1946, 1948, 1949) have published a series of informative papers on the genetics of Glomerella and on methods of handling this fungus in the laboratory. Much of their technique and some of their strains of Glomerella have been used during the present investigation.

### *Strains of Glomerella*

When EDGERTON'S plus B strain of Glomerella was crossed with the strain first obtained as a contaminant on an agar plate, the progeny segregated into four types. These four kinds of segregants were obtained both by isolating individually the eight ascospores from a single ascus and by isolating colonies at random from plated out ascospores. These four types, when grown on a complete medium,[2] are distinguishable by the different degrees of black pig-

| | | | |
|---|---|---|---|
| [2] $NH_4$ tartrate | 5 grams | glucose | 20 grams |
| $NH_4NO_3$ | 1 gram | agar | 15 grams |
| $KH_2PO_4$ | 1 gram | yeast extract | 5 grams |
| $MgSO_4 \cdot 7H_2O$ | 0.5 gram | malt extract | 5 grams |
| NaCl | 0.1 gram | $H_2O$ | 1000 ml |
| $CaCl_2$ | 0.1 gram | | |

mentation of the mycelium. Evidently, two pairs of genes are responsible for these pigment differences. If these pairs of genes are designated $A$, $A'$ and $B$, $B'$, then $AB$ is very black, $AB'$ is gray (EDGERTON's plus B), $A'B$ is also gray though easily distinguished from $AB'$, and $A'B'$ is much whiter than the others although it produces some black pigment. Gene $A$ or a closely linked gene also controls the formation of perithecia, only conidia being produced by strains having its allele $A'$ (cf. CHILTON, LUCAS, and EDGERTON 1945). This genetic analysis was further confirmed by crossing strain $AB'$ with strains $A'B'$ and $AB$. In crosses of this kind only the parental types were found among the offspring, thus indicating a single gene difference between these types.

Previous investigators (EDGERTON 1914) have used the terms plus and minus to designate the mating types of Glomerella. These designations now seem inappropriate, however, in view of the fact that crosses occur not only between plus and minus types but also between two plus types and between two minus types. Such crosses, although very infertile, were clearly demonstrated by using different pigment mutants of the same mating type. The occasional perithecia which formed along the line of contact between colonies of the two different pigment mutants frequently contained ascospores which produced the double mutant or standard (wild) type colonies. Thus crossing between the two strains must have occurred. In general, the only requirement for crossing between related strains of Glomerella is that at least one of the partners to the cross must carry the factor ($A$) for perithecia formation. However, the degree of fertility of the cross depends upon other genetic factors—a subject which will be dealt with more extensively in a later paper.

### *Induction of Mutations in Glomerella*

Since tyrosinase catalyzes at least two steps in the formation of the black pigment of Glomerella, it seemed reasonable that mutations affecting tyrosinase would be manifested as alterations in the quantity or quality of the pigment formed. This belief was reinforced by the knowledge that the formation of melanin pigments in other organisms is controlled by a large number of different genes.

Numerous mutant strains of Glomerella were obtained by irradiating the uninucleate conidia of strain $A'B$ with ultraviolet light. The conidia were obtained from cultures grown on 2 percent Difco corn meal agar or upon WESTERGAARD's (1947) synthetic medium, since the complete medium is very unfavorable for spore formation. The irradiated spores were then plated out in serial dilution cultures, and after small colonies formed, mycelial transfers were made to agar slants of complete medium. Within a few days the mycelia become large enough so that changed morphological and pigmentation characters may be easily recognized. With optimal doses of ultraviolet irradiation the number of visible mutants was usually between 15 and 30 percent of all the colonies. Although this percentage is satisfactorily high, a technique was developed for increasing the percentage of mutants. This technique was based on the fact that some irradiated spores germinate later than others, possibly because of greater absorption of the ultraviolet irradiation. Among the colonies

which germinate late, the percentage of mutants is distinctly higher than among those which germinate early. Consequently, the irradiated spores were allowed to develop in a liquid nutrient solution for periods of time ranging up to 48 hours. The mycelia of the germinated spores were then filtered off through cotton and the remaining ungerminated spores plated out. By this treatment the percentage of mutant colonies among those picked was generally raised to more than 50 percent.

*Preparation and Measurement of Tyrosinase in Glomerella Mutants*

Nutritive and other environmental conditions under which the fungus is grown exert significant effects upon the tyrosinase activity of the mycelium. Therefore when comparisons were to be made between strains, the cultures were always prepared in the same way and grown under standard conditions. The cultures were initiated by small mycelial transfers to the center of an agar slant containing 8 ml of complete medium and then grown for 8 days at 25°C. The mycelium, which forms a dense felt-like mat on the surface of the agar, was easily harvested by peeling it away from the agar substratum and scraping off any adhering pieces of agar with a dull scalpel. Enzyme extractions were made by thoroughly macerating the frozen mycelium in a homogenizer with sand, using 10 ml of phosphate buffer (pH 7.0) for each gram (wet weight) of the mycelium. The enzyme solution was then filtered and diluted, if necessary, to bring the activity within a convenient range for measurement. The temperature of the enzyme solution, during the preparative procedure, was at all times kept several degrees below room temperature. Although this procedure is subject to some uncontrolled variations, the results were essentially the same in repetitions of the experiments. Moreover, the fluctuations in activity which were observed in different enzyme preparations from any one mutant were not of the same order of magnitude as the differences between the mutants reported here.

Tyrosinase activity in the various mutant strains of Glomerella was determined by two methods: by measurements of 1) the rate of pigment (hallachrome) formation and 2) the rate of oxygen uptake during the enzymatic oxidation of tyrosine and dopa. The colorimetric determination of tyrosinase activity was made in a Klett-Summerson colorimeter using .02 M tyrosine and .02 M dopa as substrates. Air was bubbled continuously through the enzyme-substrate mixtures with high activity except for the few seconds required to make readings. Readings were taken every minute during the first ten minutes of the reaction and every five or ten minutes thereafter until the rate of the reaction leveled off (fig. 1). For preparations with low enzyme activity readings were taken only at five or ten minute intervals and continuous aeration was not provided. Comparisons of enzyme activity (table 1) were made by comparing the averages of the highest rates of activity per minute achieved in triplicate determinations with each preparation.

A less sensitive but more precise determination of tyrosinase activity was obtained by measuring the initial rate of oxygen uptake in a Warburg constant

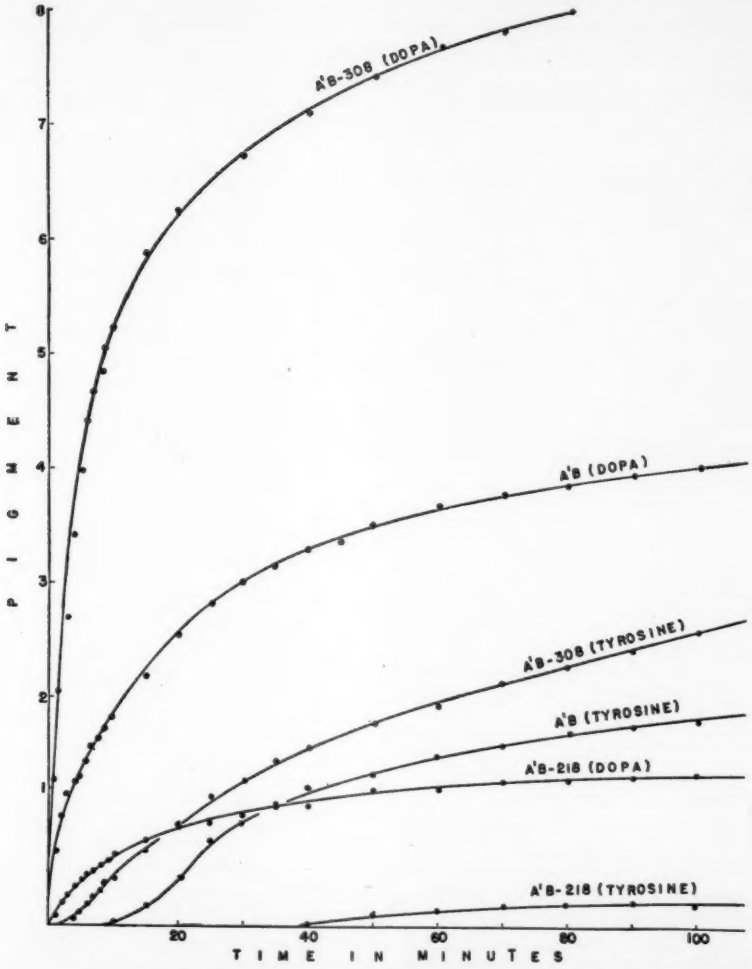


FIGURE 1.—Curves showing pigment formation during the oxidation of dopa or tyrosine at room temperature and pH 7.0 as catalyzed by tyrosinase from three strains of Glomerella. Two ml of tyrosinase solution were used for each .2 ml of .02 M substrate. The numerical values for pigment are readings $\times 10^{-3}$ taken with a Klett-Summerson colorimeter equipped with a blue filter. The curves level off because of enzyme inactivation. The enzyme preparations from *A'B*-308 were diluted to one tenth the original strength to bring the activity within the scale of the graph.

volume respirometer. The Warburg flasks, of about 15 ml capacity, contained .2 ml of 10 percent KOH in the center well, .2 ml of .02 M substrate (tyrosine or dopa) in the side arm, and 2 ml of the enzyme solution in the main part of the flask. After temperature equilibration was reached at 30°C, the reaction was initiated by tipping the substrate from the side arm into the flask. Readings of $O_2$ uptake were taken at 2 minute or 5 minute intervals depending upon the length of time the oxygen uptake curve remained linear. When dopa was

TABLE 1

*Colorimetric comparison of tyrosinase activity in extracts from mutant strains of Glomerella.*

| GENETIC CONSTITUTION OF STRAINS* | PIGMENT FORMATION PER MINUTE** | | RATIO OF ACTIVITIES DOPA/TYROSINE |
|---|---|---|---|
| | WITH DOPA | WITH TYROSINE | |
| *A'B* | 720 | 60 | 12:1 |
| *AB* | 2,000 | 90 | 22:1 |
| *AB'* | 3,400 | 175 | 19:1 |
| *A'B'* | 675 | 25 | 27:1 |
| *AB*-203 | 70 | <1 | — |
| *AB'*-203 | 202 | 6 | 34:1 |
| *A'B*-203 | 13 | 0 | — |
| *A'B'*-203 | 1 | 0 | — |
| *AB*-507 | 73 | 2 | — |
| *AB'*-507 | 4 | 0 | — |
| *A'B*-507 | 7 | 0 | — |
| *A'B'*-507 | 8 | 0 | — |
| *A'B*-200 | 32 | <1 | — |
| *A'B*-206 | 15 | 0 | — |
| *A'B*-209 | 61 | 5 | 12:1 |
| *A'B*-212 | 24 | <1 | — |
| *A'B*-216 | 29 | <1 | — |
| *A'B*-218 | 85 | 7 | 12:1 |
| *AB*-228 | 98 | 2 | — |
| *A'B*-300 | 4,440 | 230 | 19:1 |
| *A'B*-305 | 5,720 | 270 | 21:1 |
| *A'B*-308 | 12,500 | 700 | 18:1 |

* The allelic pairs *A*, *A'* and *B*, *B'* were present in the original strains. The numbered genes are mutant genes induced by ultraviolet irradiation.

** The numerical values for pigmentation are based on readings made with a Klett-Summerson colorimeter equipped with a blue filter. No ratio of activities is listed for preparations with very low activities since the calculated ratio in such instances is not accurate enough to be significant.

used as a substrate for the crude enzyme preparations, the rate of the reaction remained essentially constant for about 10 minutes in most cases (see fig. 2). When tyrosine was the substrate there was a lag period ranging up to 20 minutes with enzyme preparations of low activity. Enzyme preparations with very high activity however exhibited no lag period, probably because of the formation of small quantities of dopa in the crude enzyme preparation, since it is known that small amounts of dopa will eliminate the lag period when added to tyrosine-tyrosinase mixtures. Comparisons of the relative activity

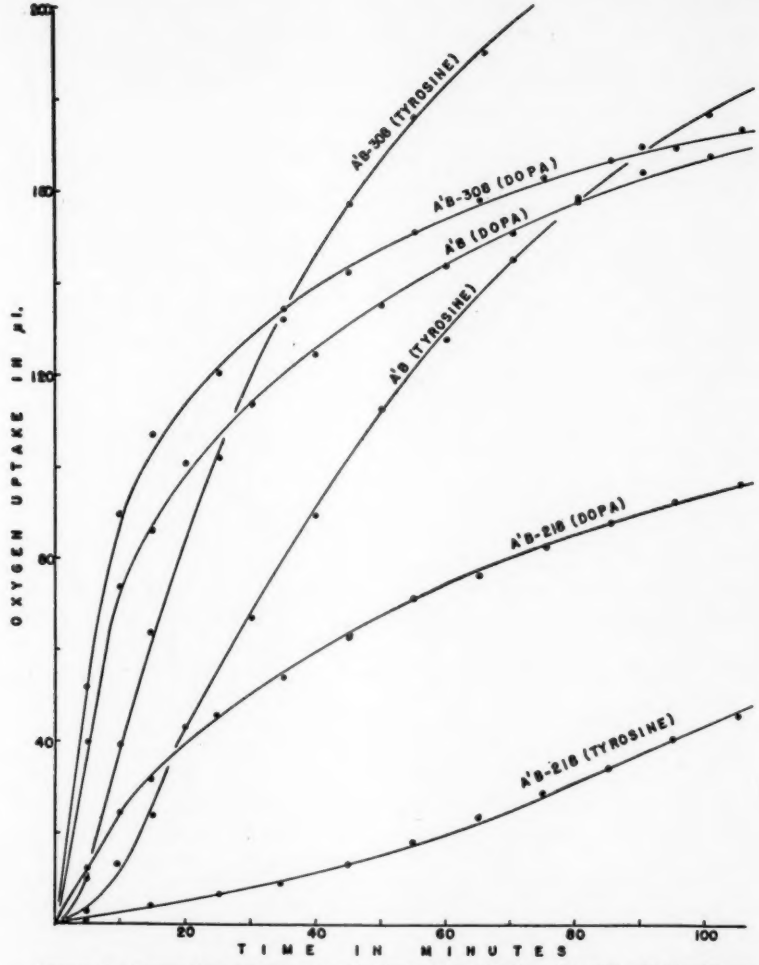


FIGURE 2.—Curves showing oxygen uptake during the oxidation of .2 ml of .02 M substrate (dopa or tyrosine) at 30°C and pH 7.0 as catalyzed by 2 ml tyrosinase solutions from three strains of Glomerella. The enzyme preparations were made in the same way except that preparations from *A'B*-308 were diluted to one tenth their original concentration to bring their activity within range of the other preparations.

of the enzyme preparations for the different mutants were made (table 2) by comparing the averages of the highest rates of activity achieved in triplicate determinations with each preparation. The initial rate of the reaction was the highest when dopa was used as the substrate, but with tyrosine as the substrate the highest rate of reaction occurred after the lag period. Highly active enzyme preparations were diluted until the maximum rate of oxygen uptake did not exceed 8μl/minute since at higher rates the oxygen supply became limiting under the conditions of the experiment.

TABLE 2

*Manometric comparison of tyrosinase activity in extracts from mutant strains of Glomerella.*

| GENETIC CONSTITUTION OF STRAINS | MICROLITERS OF OXYGEN UPTAKE PER 10 MINUTES* | | RATIO OF ACTIVITIES DOPA/TYROSINE | COMPARATIVE ACTIVITY BASED ON *A'B* AS 100 | |
|---|---|---|---|---|---|
| | WITH DOPA | WITH TYROSINE | | WITH DOPA | WITH TYROSINE |
| *A'B* | 74 | 33 | 2.2 | 100 | 100 |
| *AB* | 212 | 40 | 5.3 | 286 | 121 |
| *AB'* | 271 | 65 | 4.2 | 366 | 197 |
| *A'B'* | 77 | 27 | 2.8 | 104 | 82 |
| *AB*-203 | 12.0 | 0 | — | 16 | 0 |
| *AB'*-203 | 45.2 | 6.1 | 7.4 | 61 | 19 |
| *A'B*-203 | 2.0 | 0 | — | 3 | 0 |
| *A'B'*-203 | 3.5 | 2.8 | 1.3 | 5 | 9 |
| *AB*-507 | 14.9 | 3.5 | 4.3 | 20 | 11 |
| *AB'*-507 | 3.0 | .9 | 3.3 | 4 | 3 |
| *A'B*-507 | 1.9 | .6 | 3.2 | 3 | 2 |
| *A'B'*-507 | 2.2 | .7 | 3.1 | 3 | 2 |
| *A'B*-200 | 11.0 | 1.4 | 7.9 | 15 | 4 |
| *A'B*-206 | 4.4 | 3.0 | 1.5 | 6 | 9 |
| *A'B*-209 | 21.5 | 7.9 | 2.7 | 29 | 24 |
| *A'B*-212 | 6.7 | 2.3 | 2.9 | 9 | 7 |
| *A'B*-216 | 4.4 | 2.0 | 2.2 | 6 | 6 |
| *A'B*-218 | 23.8 | 6.5 | 3.7 | 32 | 20 |
| *AB*-228 | 12.4 | 3.5 | 3.5 | 17 | 11 |
| *A'B*-300 | 688 | 148 | 4.6 | 930 | 449 |
| *A'B*-305 | 776 | 160 | 4.9 | 1049 | 485 |
| *A'B*-308 | 1040 | 511 | 2.0 | 1405 | 1549 |

* Rates listed are averages of the highest rates achieved in triplicate determinations with each preparation. Rates greater than 80 were obtained by multiplying the measured rate by the dilution factor.

MILLER and DAWSON (1941) have pointed out the inadequacies of the manometric technique in measuring tyrosinase activity and have developed a more accurate chronometric technique based on the time required for the oxidation of a given amount of ascorbic acid by the ortho-quinone formed during the enzyme reaction. During the present investigation the manometric technique was preferred because of the possibility that the crude enzyme preparations from the different mutants might contain significantly different

amounts of compounds which might be oxidized by the orthoquinone. Such reducing substances would probably not affect the initial oxygen uptake significantly although they could greatly change the results obtained by the chronometric method.

## RESULTS

One large class of mutants, obtained by the ultraviolet irradiation of conidia, exhibited different kinds or degrees of melanin pigmentation ranging all the way from black through various shades of gray, brown, red and yellow to white. Only the mutants of the black-gray-white series are dealt with in this paper. In this series of mutants the amount of black pigment in the mycelium was usually a fairly good indicator of the tyrosinase activity found in a water extract of the macerated mycelium. Although the mutants listed in tables 1 and 2 exhibit striking differences in tyrosinase activity, it should be noted that a consideration of all the strains so far tested indicates an almost continuous gradation of activity from mutant *A'B*-507 which has the least activity to mutant *A'B*-308 which has the highest activity. The diminished tyrosinase activity of some of the mutants, particularly as measured by pigment formation, might conceivably be due to the production of inhibitors of tyrosinase rather than to a lowered production of tyrosinase itself. In fact several investigators have shown that pigment formation in crude tyrosinase-substrate mixtures may be influenced by a number of compounds independently of the enzyme concentration. Therefore, enzyme preparations from strains showing conspicuous differences of activity were mixed in an effort to discover any pigment-inhibiting effects in the preparation with low activity. Such mixtures exhibited no more than a dilution effect on the preparation with the high activity, regardless of whether pigment formation or oxygen uptake was used as a measure of enzyme activity. Thus it seems likely that the different amounts of tyrosinase activity in the various mutants represent real differences in enzyme concentration.

From tables 1 and 2 it may be seen that several enzyme preparations with very low activity on dopa have no recorded activity on tyrosine. These data alone might lead to the conclusion that several mutant genes in Glomerella result in the inability of the fungus to synthesize a true tyrosinase. However, colorimetric assays for enzyme activity on tyrosine have given indications of at least very small amounts of tyrosinase activity in each of these mutants.

Particularly noteworthy are strains *AB'*-203, *A'B*-200, and *AB*, which, in comparison to other strains, exhibit markedly different ratios of activities on the two substrates tyrosine and dopa. Although the presence of many mutant genes can be correlated with great fluctuations in the tyrosinase activity of Glomerella, with few exceptions the ratio of activities remains fairly constant. The exceptions may represent changes in enzyme specificity or may be due to closely associated metabolic products which depress one activity more than the other. On the other hand, this change in the ratio of activities may indicate that tyrosinase is indeed two enzymes. However, the fact that the great majority of the mutants with changed amounts of tyrosinase activity exhibit

about the same ratio of activities as the standard (wild) type strongly supports the view that tyrosinase is a single protein complex with two different catalytic activities.

A few of the pigment mutants tested for enzyme activity were also biochemical mutants which in addition exhibited changed morphological characters. Especially interesting are mutants 203 and 507. Although the standard type does not require any amino acid or vitamin for growth these two mutants require tryptophane. They are conspicuously different in tyrosinase activity, morphology, fertility, and even in their tryptophane requirement. Neither will grow on anthranilic acid nor on indole—precursors of tryptophane in Neurospora. In fact, they both accumulate indole or some very similar compound as indicated by the odor of the medium on which they have grown. In addition, a chemical test for indole (FEARON 1944) gave a strong positive reaction with the medium from cultures of 507 and a somewhat weaker reaction with the medium from cultures of 203. Quantitative colorimetric measurements of the indole accumulated in the medium of cultures of *A'B*-507 indicated a concentration of indole of about .01 mg per ml of medium. Medium on which the wild type has grown gives a negative result with the indole test. The fact that 507 exhibits no growth whatever in the absence of tryptophane, while 203 will grow to a very slight extent on a minimal medium is perhaps correlated with the different degrees to which they accumulate indole. Moreover, strain 507 is completely self-sterile, whereas strain 203 produces a limited number of conidia. The mutant characteristics of 507 and 203 are transmitted as a unit and are presumably due to a single gene. Strain 507, in particular, must be due to a single gene change because it reverts to the standard type at a very high rate. Thus a single gene change appears to be responsible for changes in tyrosinase activity, tryptophane requirement, pigmentation, morphology, and fertility.

The accumulation of indole by mutants 203 and 507 suggested the possibility that the observed changes in tyrosinase activity might be due to the abnormal concentration of this intermediate metabolite. Therefore tubes of complete medium were made up with .05 mg of indole per ml of medium—a concentration of indole five times as great as that found in the mutant cultures. When strain *A'B* was grown on this indole-enriched medium, the mycelium developed less pigment and contained only about 50 percent of the normal tyrosinase activity. By comparison, the presence of mutant gene 507 reduces the tyrosinase activity almost to zero. It seems unlikely, therefore, that the accumulation of indole by strains 203 or 507 can alone account for their greatly diminished tyrosinase activity. Whatever effect indole may have it appears to be exerted on the production of tyrosinase rather than on the activity of the enzyme itself. This was shown by the addition of indole to a standard tyrosinase-substrate mixture. The presence of the indole did not significantly alter the rate of pigment formation as compared to an equivalent tyrosinase-substrate mixture without indole. This influence of indole on tyrosinase production gives some additional support to the view that the various

changes in tyrosinase activity induced by mutation are due to the synthesis of different amounts of tyrosinase by the mutants rather than to the presence of inhibitors of tyrosinase.

### *Genetics of Glomerella Mutants*

The single gene nature of the hereditary changes responsible for the enzyme differences shown in tables 1 and 2 was established by the usual technique of crossing the mutant to the standard (wild) type. In a few instances the eight spores from a single ascus were isolated by free hand. In all cases the spores proved to be either four mutant and four standard, or all eight were alike. In other words, the characteristics of each mutant were always inherited as a group as if they were due to a single gene. Where the eight spores were found to be alike it seemed reasonable to assume that no crossing had occurred, particularly in view of the fact that perithecial strains form ascospores homothallically. Of course, what appear to be unit factors may be closely linked groups of factors. Only data from very large numbers of offspring can render this possibility implausible. Since direct manual isolation of single spores is very time-consuming, a simpler procedure was used in testing large numbers of spores for evidence of crossing over. Perithecia which were formed by crossing between the standard type and the mutant were crushed on a glass slide. The ascospores were then pipetted off and plated out in dilution cultures. After the spores germinated, mycelial transfers were made to agar slants. In this way the genetic constitution of several hundred spores was easily studied. For each mutant strain listed in tables 1 and 2, at least a hundred colonies which had segregated from a cross with the standard type were examined. In the case of some mutants several hundred cultures were examined, but in all instances the mutant character was inherited as if due to a single gene. Some cultures were found to be double mutants by this technique but none of these double mutants is listed in table 1 or 2.

The non-allelic nature of the mutants was shown by forming double mutants or by segregating out the wild type from a cross between two mutants. In some fungi, such as Neurospora, the formation of heterocaryons may also be used as a test for allelism. However, the few attempts which were made to form heterocaryons in Glomerella were unsuccessful as one would expect if the hyphal cells are unicellular (ULLSTRUP 1938; WHEELER, OLIVE, ERNEST, and EDGERTON 1948). All the mutant genes discussed here have not yet been tested for allelism. However, genes *A* and *B* have been shown to be non-allelic with each other and with all the other genes in tables 1 and 2. Thus from these data alone at least three loci are involved in the control of tyrosinase activity. Moreover, four additional non-allelic genes (not included in the tables) have been shown to affect both tyrosinase activity and the color of the mycelium. Therefore, at the present time at least six different loci have been shown to affect tyrosinase activity, and it is presumed that most, if not all, of the remaining mutants also represent distinct loci. It seems reasonable to conclude on the basis of these data that tyrosinase activity in Glomerella is affected by many different genes

which probably act by altering the normal balance of biochemical reactions in such a way as to reduce or increase the synthesis of tyrosinase.

The complexity of the genetic control of tyrosinase synthesis is illustrated by the different effects produced by various combinations of mutant genes. For example genes 203 and 507 have in common the property of preventing the synthesis of tryptophane and also of greatly diminishing the production of tyrosinase. However, these genes may be readily distinguished by the different effects produced in combination with the allelic pairs $A$, $A'$, and $B$, $B'$. The strain $AB'$-203 has nearly four times the tyrosinase activity of $AB$-203 (see table 2). By contrast, strain $AB'$-507 has only 1/5 as much tyrosinase activity as $AB$-507. Thus gene $B$ is more effective than its allele $B'$ in promoting tyrosinase synthesis in the presence of mutant gene 507, whereas the allele $B'$ is more effective than gene $B$ in the presence of gene 203. In combination with $A'B$ or $A'B'$ both 507 and 203 diminish tyrosinase activity almost to the vanishing point. It is interesting to note that neither gene $A$ nor $B$ nor their alleles $A'$, $B'$ exert any effect upon the tryptophane requirement induced by the presence of either gene 203 or 507.

## DISCUSSION

The data obtained in this investigation indicate that the synthesis of the enzyme tyrosinase is under the control of a large number of different genes. The mechanism of this control, however, still remains obscure. Whatever the mechanism of control may be, it must be able to produce a finely graded series of quantitatively different effects in order to explain the gradations of tyrosinase activity in Glomerella mutants. Quantitative variations in the production of essential growth substances may also be observed among the biochemical mutants of Glomerella and Neurospora, although more attention has been given to those mutants which produce very little, if any, of some essential growth factor. In explanation of the biochemical mutants of Neurospora the hypothesis has been advanced (BEADLE 1945) that the gene impresses upon the enzyme molecule that specific configuration which determines its catalytic capacities. This hypothesis explains very well a graded series of quantitative effects produced by a series of allelic genes, since allelic genes could impress configurations of different catalytic efficiencies upon the enzyme molecule. Similarly, different quantities of a single enzyme produced through the cooperative activity of many non-allelic genes can also be attributed to an altered efficiency in carrying on any one of the gene-controlled contributory reactions. Thus the data on tyrosinase activity in Glomerella mutants do not contradict the one gene-one specific enzyme hypothesis. Neither do the data contradict the hypothesis that the specificity of an enzyme is the logical culmination of its characteristic synthesis, in which case there would be no single "tyrosinase" gene acting like a template to impress a specific catalytic configuration upon an already formed protein molecule. Further studies with purified preparations of tyrosinase from the Glomerella mutants exhibiting different ratios of activities may provide critical data for evaluating these two

hypotheses. The admittedly inadequate data from the crude enzyme preparations do suggest the possibility, however, that genetically-induced alterations in the metabolic pattern of the cell may qualitatively change the nature of the enzyme formed. In this connection it is interesting to note the work of CASPARI and RICHARDS (1948) on the proteins of $a^+a^+$ and *aa* Ephestia moths. The mutant Ephestia, which is unable to transform tryptophane into kynurenine, has a measurably higher tryptophane content than the wild type. The authors conclude from their measurements that part of the increased tryptophane content of *aa* Ephestia is stored in the proteins; thus, the normal protein make-up of the cells must be changed.

It is generally assumed that an additional growth factor requirement in a mutant strain of Glomerella or Neurospora is due to the absence (or very low activity) of an enzyme which catalyzes some step in the synthesis of the growth factor. This assumption implies that in the case of the tryptophane-requiring, essentially tyrosinaseless mutants *A'B*-203 and *A'B*-507 a single gene change has effectively prevented the synthesis or activity of two distinct enzymes (cf. HOROWITZ 1949, for discussion of number of functions per gene). Furthermore, this double effect does not appear to result from any simple inhibiting action of accumulated precursors of tryptophane on either the synthesis or activity of tyrosinase as was shown by the addition of indole and anthranilic acid to the medium of standard cultures. The absence of inhibiting precursors is further suggested by the fact that genetic changes can significantly increase tyrosinase synthesis without affecting the tryptophane requirement. Of course, such genetic changes might conceivably act by removing some inhibiting precursor of tryptophane synthesis.

It should be noted again that the amount of tyrosinase in the mycelium varies greatly with the age of the mycelium and with the conditions of nutrition, aeration, and temperature under which it is grown. Such great variability in tyrosinase content indicates that the enzyme is of no great importance to the life of the fungus. It is perhaps not surprising, therefore, that genetically-induced changes in the normal biochemical pattern of the cell should also result in great fluctuations in the synthesis of tyrosinase. In other investigations of biochemical genetics moreover, it is also quite possible that the synthesis of other enzymes is similarly dependent upon the metabolic pattern of the cell, and therefore, correlations of genetic constitution with enzyme activity are not conclusive demonstrations of any specific, direct relationship between the gene and the enzyme.

In many ways the polygenic control of tyrosinase activity seems very similar to the action of the numerous suppressor and enhancer genes found in Drosophila (BRIDGES and BREHME 1944) and in Neurospora (HOULAHAN and MITCHELL 1947; EMERSON 1948), since enhancer and suppressor genes are likewise non-allelic genes affecting the same character. However, in Drosophila and Neurospora the various suppressor and enhancer genes for any one character have not yet been shown to act through a single enzyme system although such a mechanism of action seems reasonable. In Glomerella, most of the genes

affecting pigmentation do act through the single mechanism of changing the concentration of tyrosinase, although it is highly improbable that the primary action of all of these genes is directly upon the enzyme.


## SUMMARY

1. The object of this investigation was to study the genetic control of enzyme synthesis. The enzyme tyrosinase, by reference to the work of other investigators, was shown to have characteristics which make it particularly favorable for a study of the relationship between genes and enzymes. Different kinds of tyrosinase are found in different organisms, and the activity of tyrosinase (or dopa oxidase) appears to be modifiable by many different genes.

2. The ascomycete, *Glomerella cingulata*, was found to be a rich source of tyrosinase. Mutant strains of Glomerella were obtained by ultraviolet irradiation of the conidia. Standard genetic procedures were used in demonstrating the non-allelic, single gene nature of the mutant strains used in this investigation. Many of these mutant strains exhibited different degrees of melanin pigmentation and contained different amounts of tyrosinase, thus demonstrating the polygenic control of tyrosinase activity.

3. Two distinct Glomerella mutants with almost no tyrosinase activity were also unable to synthesize tryptophane, presumably because of the absence of an enzyme essential for tryptophane synthesis. In these mutants a single gene change apparently controls the activity of two distinct enzymes.

4. The many genes influencing melanin pigmentation in Glomerella resemble, in their mode of action, the suppressor and enhancer genes described in other organisms. Although the genetically-induced changes in pigmentation in Glomerella are usually correlated with changes in tyrosinase activity, it is highly improbable that the primary effect of these genes is directly upon tyrosinase itself.



## ACKNOWLEDGMENTS

The use of Glomerella for the study of gene-enzyme relationships was first begun at JOHNS HOPKINS UNIVERSITY in collaboration with SOL H. GOODGAL whose stimulating contributions to the inception of this work are gratefully acknowledged. The author is also greatly indebted to the staff of the biology division at the CALIFORNIA INSTITUTE OF TECHNOLOGY for their intellectual and material aid during the course of this investigation.


## LITERATURE CITED


BEADLE, G. W., 1945 Biochemical genetics. Chem. Rev. **37:** 15–96.
1948 Physiological aspects of genetics. Ann. Rev. Physiol. **10:** 17–42.

BRIDGES, C. B., and K. S. BREHME, 1944 The mutants of *Drosophila melanogaster*. Carnegie Instn. Wash. Publ. 552: 253 pp.

CASPARI, E., and JOSEPHINE RICHARDS, 1948 On the proteins of $a^+a^+$ and $aa$ Ephestia. Proc. nat. Acad. Sci., **34:** 587–594.

CHILTON, S. J. P., G. B. LUCAS, and C. W. EDGERTON, 1945 Genetics of Glomerella. III. Crosses with a conidial strain. Amer. J. Bot. **32:** 549–554.

CHILTON, S. J. P., and H. E. WHEELER, 1949 Genetics of Glomerella. VI. Linkage. Amer. J. Bot. **36:** 270–273.

DALTON, H. R., and J. M. NELSON, 1939 Tyrosinase from the wild mushroom, *Lactarius piperatus*. J. Amer. chem. Soc. **61**: 2946–2950.

EDGERTON, C. W., 1914 Plus and minus strains in the genus Glomerella. Amer. J. Bot. **1**: 244–254.

EDGERTON, C. W., S. J. P. CHILTON, and G. B. LUCAS, 1945 Genetics of Glomerella. II. Fertilization between strains. Amer. J. Bot. **32**: 115–118.

EMERSON, STERLING, 1948 A physiological basis for some suppressor mutations and possibly for one gene heterosis. Proc. nat. Acad. Sci. **34**: 72–74.

FEARON, W. R., 1944 The detection of indole by means of xanthydrol. The Analyst **69**: 122–123.

GINSBURG, B., 1944 The effects of the major genes controlling coat color in the guinea pig on the dopa oxidase activity of skin extracts. Genetics **29**: 176–198.

GINSBURG, B., and F. KAPLAN, 1947 Evidence for the presence of a gene controlled oxidase in mouse skin extracts. Genetics **32**: 87–88 (abstract).

HALDANE, J. B. S., 1942 New Paths in Genetics. 206 pp. New York and London: Harper and Brothers.

HOROWITZ, N. H., 1949 Biochemical genetics of Neurospora. Advances in Genetics. (In press.)

HOULAHAN, M. B., and H. K. MITCHELL, 1947 A suppressor in Neurospora and its use as evidence for allelism. Proc. nat. Acad. Sci. **33**: 223–229.

KASTLE, J. H., 1910 The oxidases and other oxygen-catalysts concerned in biological oxidations. Hygienic Laboratory Bull. 59 (U. S. Treasury Dept.)

LUCAS, G. B., 1946 Genetics of Glomerella. IV. Nuclear phenomena in the ascus. Amer. J. Bot. **33**: 802–806.

LUCAS, G. B., S. J. P. CHILTON, and C. W. EDGERTON, 1944 Genetics of Glomerella. I. Studies on the behavior of certain strains. Amer. J. Bot. **31**: 233–239.

MILLER, W. H., and C. R. DAWSON, 1941 A new method for the measurement of tyrosinase catecholase activity. J. Amer. Chem. Soc. **63**: 3375–3382.

MITCHELL, H. K., and J. LEIN, 1948 A Neurospora mutant deficient in the enzymatic synthesis of tryptophane. J. biol. Chem. **175**: 481–482.

NELSON, J. M. and C. R. DAWSON, 1944 Tyrosinase. Advances in Enzymology **4**: 99–152.

RUSSELL, L. B., and W. L. RUSSELL, 1943 A study of the physiological genetics of coat color in the mouse by means of the dopa reaction in frozen sections of skin. Genetics **33**: 237–262.

SAWIN, P. B., and D. GLICK, 1943 Atropinesterase, a genetically determined enzyme in the rabbit. Proc. nat. Acad. Sci., **29**: 55–59.

SCHUURMAN, J. J., 1937 Contributions to the genetics of *Tenebrio molitor* L. Genetics **19**: 273–355.

ULLSTRUP, A. J., 1938 Variability of *Glomerella gossypii*. Phytopathology **28**: 787–798.

WESTERGAARD, M., and H. K. MITCHELL, 1947 Neurospora V. A synthetic medium favoring sexual reproduction. Amer. J. Bot. **34**: 573–577.

WHEELER, H. E., and S. J. P. CHILTON, 1948 Multiple alleles and factor interaction in Glomerella. Phytopathology **38**: 28 (abstract).

1949 Production of mutants in Glomerella as influenced by age of culture. Phytopathology **39**: 25 (abstract).

WHEELER, H. E., L. S. OLIVE, C. T. ERNEST, and C. W. EDGERTON, 1948 Genetics of Glomerella. V. Crozier and ascus development. Amer. J. Bot. **35**: 722–728.

WINGE, Ö., and CATHERINE ROBERTS, 1948 Inheritance of enzymatic characters in yeasts, and the phenomenon of long-term adaptation. C. R. Lab. Carlsberg, Serie physiologique **24**: 263–315.

WRIGHT, S., 1941 The physiology of the gene. Physiol. Rev. **21**: 487–527.

1942 The physiological genetics of coat color of the guinea pig. Biol. Symp. **6**: 337–355.

# ADDITIONAL GENE-CONTROLLED ANTIGENIC FACTORS IN THE BOVINE ERYTHROCYTE[1]

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Received June 6, 1949

IN PREVIOUS reports from this laboratory evidence has been presented for the existence of 30 gene-controlled antigenic factors in the erythrocytes of cattle (FERGUSON 1941; FERGUSON, STORMONT and IRWIN 1942). Each of these 30 antigens of cattle in contrast to its absence has behaved as a dominant character, as do the known antigenic components of blood cells in other species. None of the 30 factors are necessary alternatives as are the M and N agglutinogens of human cells (LANDSTEINER and LEVINE 1928). Evidence for antibodies which detect additional antigenic variation in the erythrocytes of cattle is given in this report. Attention was previously called to the associations of certain of these antigenic factors with others (FERGUSON 1941; STORMONT, IRWIN and OWEN 1945). These relationships are somewhat comparable to the relationships of the so-called subtypes $A_1$ and $A_2$ of the A agglutinogen of man. A system of designating serological subtypes of certain of these antigenic components is proposed.

## MATERIALS AND METHODS

The 30 previously described antigens of cattle erythrocytes were assigned symbols (A, B, C, E, G, H, I, J, K, M · · · Z, A′, C′ · · · H′) in the order of their discovery. No relationship between A and A′, C and C′, etc. is implied in this system. As may be noted, the letters D, F, L and B′ have not been used. The antigens which at one time were called D and F were found to be the same as those called B and C, respectively. Likewise, the designations L and B′ were used only temporarily. The letter F has now been assigned to a newly discovered antigenic property of cattle cells described herein.

The method of preparing "reagents" (antibodies) which detect presumably unit-antigens in the red blood cells of cattle was described in detail by FERGUSON (1941) and illustrated pictorially by STORMONT and CUMLEY (1943). In general, this consisted in the production of iso-immune sera in cattle and antisera in rabbits against the erythrocytes of selected donors. These more or less complex antisera (hemolysins) were fractionated into individual reagents by absorption with the reactive cells of selected cattle. Each fraction of antibody which behaved as if it were not further divisible was tested with the bloods of 20 or more cattle whose antigenic formulas (blood types) up to that time were

[1] From the Departments of Genetics (No. 407), Dairy Husbandry and Veterinary Science, College of Agriculture, UNIVERSITY OF WISCONSIN, in cooperation with the Bureau of Animal Industry, U. S. DEPARTMENT OF AGRICULTURE. This project is part of a program formerly aided by grants from the HOLSTEIN-FRIESIAN ASSOCIATION OF AMERICA and the AMERICAN GUERNSEY CATTLE CLUB.

TABLE 1

*Blood types of 40 cattle of the experimental herd.**

| NO. | BLOOD TYPES** |
|---|---|
| 1Z | AB–F–H–J–MNO$_1$–––––U$_1$––X$_2$Y$_1$ZA′–E$_2$′F′G′H′–––– – |
| 2Z | –BC$_1$FG–––KMNO$_2$–––S––VW–Y$_1$–A′–E$_2$′–G′H′I′––L′– |
| 3Z | ––C$_1$F–H–J–––O$_2$–––––V–––––––F′–––J′K′–– |
| 4Z | ABC$_2$FG—JK––O$_1$–––––VWX$_2$Y$_1$ZA′–E$_2$′–G′H′I′–K′–– |
| 6Z | –––F–—J–––O$_1$P––––––––Y$_1$Z––E$_2$′––H′I′–––– |
| 7Z | A––FGH–––––O$_2$–Q–––––––X$_2$Y$_2$Z–D′E$_1$′F′G′H′–––– |
| 8Z | A–C$_1$F–––––––O$_1$–––––––––––D′–F′–H′I′J′––– |
| 9Z | A–C$_1$–G–––––––O$_1$–––ST$_1$–VWX$_1$Y$_2$Z––E$_1$′F′G′H′I′–––– |
| 10Z | A–C$_1$F–H–––––O$_1$–––––––––Z–––F′–H′I′––––– |
| 11Z | A––FG––––––––Q––––––WX$_2$Y$_1$Z–D′–––––––– |
| 12Z | AB–F–––J–––O$_1$–––S–––WX$_2$Y$_1$–––E$_2$′–G′H′–––––– |
| 13Z | –B–––GH–J–––O$_1$–––––V X$_2$Y$_1$––––F′–––––L′– |
| 14Z | A–C$_1$F–––––MNO$_2$ ––––––VWX$_2$Y$_1$Z–––––H′I′J′K′–– |
| 16Z | –B FG––JK––O$_2$–––––VW–Y$_1$ZA′–E$_1$′–G′–––K′–– |
| 17Z | A––F–––––––O$_2$P–––––WX$_2$–Z–D′E$_2$′–G′H′I′–––– |
| 18Z | AB–F–H––––NO$_1$––R–––WX$_2$–ZA′D′––G′H′––––– |
| 19Z | AB–F–––––––O$_1$–––––U$_2$–––Z–––F′–––––––– |
| 20Z | ABC$_1$FG––J–MNO$_2$–––––––X$_1$Y$_1$–A′–E$_1$′F′–H′––––– |
| 21Z | A–C$_1$FG–I ––––––––––––Y$_2$Z–––F′–H′I′––L′Z′ |
| 22Z | A–C$_1$F–––––––O$_1$–––––U$_1$–WX$_1$–Z–D′E$_1$′––H′–––– |
| 23Z | ABC$_1$FG–––––––O$_1$––RS–––WX$_1$–––––G′H′––––– |
| 24Z | ––C$_1$F–––J–MNO$_2$–––––––––Z–––F′–H′–J′K′–– |
| 25Z | A–– F–H–J–––O$_2$–––––––X$_2$Y$_2$Z–D′E$_1$′–G′H′–J′K′–– |
| 26Z | ABC$_2$F–H–––––O$_1$–R–––V–X$_2$–Z––E$_2$′––H′––––– |
| 28Z | A–C$_2$F–H–––––––––––––Z––E$_2$′F′———–––– |
| 29Z | ––– FG––J–MNO$_2$–––S–––WX$_2$Y$_2$Z–D′E$_1$′F′–H′––––– |
| 30Z | ––C$_1$F––––MNO$_2$–––––V–X$_2$Y$_1$ZA′———H′–J′K′L′– |
| 31Z | ABC$_1$FG––JK–NO$_1$ ––––––––X$_2$Y$_1$–A′D′E$_2$′F′G′H′I′–––– |
| 32Z | ABC$_1$FG–IJ–MNO$_1$–––ST$_2$–––X$_2$–ZA′–––G′H′––––– |
| 33Z | –B–F–––––––O$_2$–––S–––VWX$_2$––A′D′E$_2$′F′–H′––––– |
| 34Z | –B–FG––K––O$_2$–––S–––VW–Y$_2$ZA′D′–F′G′H′––––– |
| 35Z | –B–FG–––––O$_2$–––S––VW–Y$_2$ZA′D′E$_1$′F′–H′––––– |
| 6g | –––F––––––NO$_1$ –––––V–X$_2$–ZA′D′E$_2$′–G′H′––––– |
| 8g | A––FG–––––––———–––––X$_2$Y$_2$——E$_1$′F′–H′––––– |
| 11g | –––FGHI–––––———–––––X$_2$Y$_2$–––E$_1$′F′–H′––––– |
| 25g | –––F–––––––O$_2$–––––V–X$_2$–––D′E$_2$′–G′H′––––– |
| 33g | –––FG––J––NO$_1$–––S–––––Y$_2$–A′–E$_1$′F′–H′––––– |
| 15E | –BC$_1$FG––J–MNO$_1$–––––––––Y$_1$ZA′D′E$_1$′F′–H′––––– |
| 20E | –BC$_1$FG––JK–––––––––––X$_1$–––D′E$_2$′–G′H′––––– |
| 1F | –BC$_1$FG––J ––––––––––––Y$_2$Z––E$_1$′F′–H′––––– |

* The blood types constitute a summary of the positive reactions of the cells of each of the 40 cattle when tested with each of 44 reagents (antibodies) described in this and previous reports (*loc. cit.*). Copies of the protocol from which these blood types were summarized are permanently filed in the Editorial Office of GENETICS.

** The antigenic factors described in this report include the factors I′, J′, K′, L′, Z′, F′ and subtypes of the factors O, T, U, X and E′. The subtype factor $C_1$ is equivalent to the two previously described factors C and E together, while subtype $C_2$ is equivalent to the C factor alone. Likewise, subtype factor $Y_1$ is equivalent to YC′, whereas $Y_2$ represents factor Y in the absence of C′.

known (see table 1). The results of such tests were usually sufficient to determine whether a reagent was a reasonable facsimile of one or more already in use, or whether the reagent was detecting a new antigenic factor.

Certain changes have been made in previously reported techniques, as follows: The volume of blood (1000 ml of citrated whole blood for each transfusion) previously used in the iso-immunizations has been reduced to 250 ml and, more recently, to only 25 ml. The smaller amounts were considered less likely to result in severe transfusion-reactions and, perhaps, to be no less effective in engendering the iso-antibodies. The pairing of animals (donors and recipients) in iso-immunizations was made on the basis of their antigenic formulas. This method allowed the prediction of at least some of the antibodies that were produced when the immunizations were effective. In addition, the absorption-analyses of the resulting antisera were facilitated. Theoretically, antibodies would be expected for the antigenic components in the blood of the donors not present in the recipients, including those not detected previously.

## EXPERIMENTAL

Table 1 shows the blood types of 40 non-registered animals of the experimental herd following tests with each of the reagents described in this and previous reports (*loc. cit.*). No two of the 44 test-fluids gave parallel reactions with the corpuscles of these 40 cattle. Copies of the protocol from which these blood types (table 1) were summarized are permanently filed in the Editorial Office of GENETICS.

### *I′*

Cow 1Z (with the antigenic formula ABHJMNOUYZA′C′F′G′H′) was immunized with the blood of 6Z (JOPYZC′H′) in anticipation of the formation of antibodies for bloods containing antigen P. (Note that the blood of the donor contained only the known antigenic component P not found in the recipient's blood.) The antiserum was collected on the sixth day following the fourth transfusion. It reacted (titer approximately 1:64) with the corpuscles of 10 of the 40 experimental animals. Among these 10 were the only two animals in the herd with antigen P (i.e., the donor 6Z and cow 17Z, table 1). Samples (diluted 1:2) of this iso-immune serum when absorbed with the cells of either the donor or cow 17Z were depleted of all demonstrable antibodies. On the other hand, reactive bloods which lacked the P factor exhausted the antibodies for this class of cells, but not those for the cells of 6Z and 17Z. These results suggested that in addition to those antibodies that reacted with bloods having factor P, the antiserum contained antibodies against a previously unrecognized antigenic component which is called I′. The association of the antigenic factor P with I′ in both the donor and 6Z proved to be no mere coincidence, since subsequent tests on the cells of several thousand cattle have shown that the comparatively rare antigenic factor P (OWEN, STORMONT and IRWIN 1947) occurs with I′ more often than could be expected by chance. Additional absorptions of this antiserum with bloods possessing the P factor,

however, demonstrated several bloods which exhausted the antibodise for P but not those for I′—such bloods contained the P factor but not I′.

The recipient (1Z) was later re-immunized (one transfusion of 250 ml of the blood of 6Z). This second antiserum contained more potent antibodies (titer >1:256) than the first. These two antisera gave parallel reactions when tested with the bloods of the 40 animals (table 1). An I′ reagent prepared from the second antiserum was further absorbed with the reactive bloods of each of 23 cattle and in each instance the demonstrable antibodies were exhausted. Conventionally, these results would imply that each of these antisera contained two major fractions of antibodies; namely, one that reacted with a previously recognized antigenic component called P and the other with the new factor I′. It is, of course, not possible to determine by these serological tests whether one or two antigenic substances in the cells of the donor were responsible for the stimulation of the antibodies. On the other hand, it is relatively certain that at least two different serologically reactive substances found in the cells of other cattle were responsible for the fractionation of the antibodies.

The protocols of the absorptions in preparation and testing this reagent as well as for all the others are not given in this report, however, these will be supplied upon request.

*J′*

Similarly, two cows, 4Z (ABCGJKOVWYZA′C′G′H′I′) and 13Z (BGHJOVYC′F′), were immunized with the blood of 3Z (CEHJVF′) in anticipation of the production of antibodies for the factors E, H and F′ in cow 4Z and those for C and E in 13Z. From each of the resulting antisera reagents were prepared which did not parallel the reactions obtained with any one of those previously described. The serological factor of the cells that reacted with these two parallel reagents is called J′. The more potent of these J′ reagents, obtained from the antiserum of 4Z (titer of reagent >1:64), was selected for further study. When this reagent was absorbed with the reactive bloods of each of seven cattle, all the antibodies were absorbed.

*K′*

In addition to J′ antibodies, a second reagent that reacted with cells possessing a new factor called K′ was obtained from the antiserum of 13Z (above). Subsequent absorptions indicated that not all reactive bloods had the same avidity for the K′ antibodies, since two bloods were encountered which failed to exhaust all the K′ antibodies. Nevertheless, all bloods which were reactive with this reagent were designated K′. The K′ reagent, although used regularly in the serological tests, has occasionally failed to elicit positive reactions with previously determined reactive cells. This in itself undoubtedly has accounted for some errors in the blood-typing studies. When this reagent was used, either freshly prepared or freshly thawed from frozen fluids, the reactions obtained were at their best; if allowed to stand at refrigerator temperatures, (±4°C) the K′ antibodies gradually lost their strength.

*L′*

An antiserum produced in 14Z (ACEMNVWYZC′H′I′J′K′) by immunizing with the blood of 30Z (CEMNVYZA′C′H′J′K′) did not contain antibodies for antigen A′ as might have been expected. However, a new reagent was prepared from this antiserum. The factor of the cells that reacted with this reagent is called L′. Absorption of this L′ reagent with the reactive bloods of each of nine cattle exhausted the antibodies in each instance.

*Z′*

An antiserum was produced in cow 38g (BCEGJKVYZA′C′D′G′H′) by immunizing with the blood of a Zebu bull, 14G (ABCEGHJQUVWXYA′F′G′). This serum contained a variety of different antibodies, and among these there was a fraction which did not parallel the reactions of any previously described. The cellular character reactive with this fraction is called Z′. The Z′ reagent reacted with the cells of only one of the 40 animals (table 1). In fact, in tests to date on the bloods of several thousand registered Holstein-Friesians, none has been found to possess this character. Z′ has been observed in Guernsey cattle; the incidence, however, was less than four percent.

*F*

The normal serum collected from 9Z (ACEGOSTVWXYZE′F′G′H′I′) at several intervals prior to immunization with the blood of 33g (GJNOSYA′E′-F′H′) had been shown to contain normal antibodies for the J factor. Immune antibodies were present in the sample of serum taken just before the second injection. These antibodies reacted with the bloods of all the 43 experimental animals except the cells of the recipient. When this antiserum was absorbed by the cells of one of these cows (13Z), only the normal antibodies for J were exhausted; those remaining reacted with the cells of all the animals (table 1) except the cells of the recipient (9Z) and cow 13Z. The letter F is assigned to this new antigenic factor.

*On the Inheritance of the Serological Characters I′, J′, K′, L′, Z′ and F*

The genetic data in table 2 show the kinds and numbers of progeny from each of the three possible kinds of matings, i.e., those in which the antigenic factor was possessed by (a) both parents, (b) only one of the parents and (c) neither parent, for each of the factors I′, J′, K′, L′ and F. For example, in 25 matings in which both parents were type I′ (i.e., I′×I′ matings) there were 25 progeny with I′ and six without. In the 76 matings in which only one of the parents posssesed I′ (i.e., matings of I′×—) there were 47 offspring with I′ and 38 without. No progeny with I′ were noted among 278 animals from 242 different matings in which the cells of neither parent reacted with the I′ reagent.

Similarly, the results on the inheritance of the remaining five factors are shown. With the exception of three apparent discrepancies (the appearance of three progeny of type K′ from matings in which the cells of both parents ap-

TABLE 2

*Inheritance of the antigenic factors I', J', K', L', Z' and F*

| FACTOR | TYPE OF MATING | NUMBER OF PROGENY | |
|---|---|---|---|
| | | POSSESSING FACTOR | LACKING FACTOR |
| I' | I'×I' | 25 | 6 |
| | I'×—* | 47 | 38 |
| | —×— | 0 | 278 |
| J' | J'×J' | 25 | 8 |
| | J'×— | 58 | 51 |
| | —×— | 0 | 278 |
| K' | K'×K' | 25 | 10 |
| | K'×— | 68 | 72 |
| | —×— | 3 (?) | 751 |
| L' | L'×L' | 7 | 1 |
| | L'×— | 27 | 37 |
| | —×— | 0 | 362 |
| Z' | Z'×Z' | 0 | 0 |
| | Z'×— | 6 | 4 |
| | —×— | 0 | 430 |
| F | F×F | 366 | 8 |
| | F×— | 7 | 6 |
| | —×— | 0 | 0 |

* The dash (—) symbolizes the absence of the factor.

peared to be non-reactive with K' antibodies), the general rule applies that an individual possessed a character only when it was present in one or both parents. Although it was not possible to re-check the blood of the parents of these three exceptional offspring, it is believed that there had been weak reactions with the K' reagent which were not detected. In other matings where the genetic results indicated that reactions had been missed, check-tests confirmed this opinion.

These data constitute evidence that the six blood factors (I', J', K', L', Z' and F) are gene controlled. Furthermore, each of these factors appears to be inherited as a simple dominant when contrasted with its absence.

### *Serological Subtypes and Cross Reactions*

#### Subtypes of T ($T_1$ and $T_2$)

Most of the reagents or antisera used in the initial characterization of the 30 antigenic components of cattle erythrocytes (*loc. cit.*), as well as those reported herein, had been repeatedly checked for divisibility into additional fractions of antibodies. Further absorptions, however, have shown that a number of these test-fluids could be subdivided. The resulting fractions have

generally reacted too weakly to be of any practical use in blood-typing, but some exceptions to this statement have been noted.

As previously reported (FERGUSON, STORMONT, and IRWIN 1942) the T reagent was produced by immunizing cow 844 with the blood of her daughter 871. This antiserum was absorbed with the reactive cells of each of 22 different individuals and the antibodies appeared to be completely exhausted in each case. It was concluded, therefore, that the antiserum contained antibodies for a single antigen called T.

Subsequently, a reagent was prepared from an antiserum produced in cow 1450 (ABCNOYA′C′G′H′) with blood transfused from cow 421 (AGTG′) which appeared to be quite similar to the original "T" reagent (anti-871). The

TABLE 3

*Subtypes of factor T*

| SUBTYPES OF T | REACTIONS WITH REAGENTS | | ABSORPTION OF ANTI-871 SERUM WITH | |
|---|---|---|---|---|
| | ANTI-871 (ORIGINAL) | ANTI-421 | $T_2$ CELLS | $T_1$ CELLS |
| $T_1$ | ++ | + | + | 0 |
| $T_2$ | + | 0 | 0 | 0 |
| —* | 0 | 0 | 0 | 0 |

* The dash (—) indicates that class of bloods which was non-reactive with either of these T reagents. (++ strong reaction; + moderate reaction.)

second T or T-like reagent (anti-421) paralleled the reactions obtained with the original T reagent in tests on the cells of Guernsey, Brown Swiss and Jersey cattle but failed to react with the bloods of certain Holstein-Friesians which were lysed by the original reagent. In addition, it was noted that the corpuscles of these Holsteins were more slowly and less completely hemolysed by the original reagent than were bloods that reacted with both these fluids. Absorption of the original antiserum with the bloods of type T that were non-reactive with the second reagent resulted in exhausting the antibodies only for cells of this type but not for those that reacted with both T reagents. Additional attempts were made to fractionate the original T reagent by absorbing with bloods that reacted with both reagents and, as in the initial absorptions (FERGUSON, STORMONT, and IRWIN 1942), all demonstrable antibodies were exhausted. Those bloods reactive with both the original or anti-871 reagent and the anti-421 reagent are now called $T_1$ to differentiate them from the bloods that reacted only with the original 'T reagent. The latter bloods are designated $T_2$. The relationship of these two serological subtypes of T is shown in table 3.

Absorption of the anti-871 reagent with bloods of subtype $T_2$ markedly reduced the titer of the antibodies that remained for bloods of type $T_1$. This same phenomenon, to a greater or lesser degree, was noted for each of the following examples of subtypes, and is considered indicative of cross reactions

of antibodies with closely related substances, as discussed later in this report.

It should be pointed out that this system of designating serological reactions of cells is an abbreviated method. For example, the subtypes $T_1$ and $T_2$ could be written $T_{1\cdot2}$ and $T_2$, respectively, since both subtypes have a factor in common but only subtype $T_1$ has factor 1. The primary reason for the use of the subtypes as proposed is the belief that reactions of this nature are indicative of overlapping relationships (cross reactions) among the serologically reactive substances of bovine erythrocytes (see discussion). The failure to depict the hypothetical factor 1 separately from 2 indicates that no more than one substance in any one blood need be postulated to account for the reactions of the bloods with both the demonstrated fractions of antibody in the anti-871 reagent. This explanation is implied in the use of the single symbol $T_1$.

### Subtypes of U ($U_1$ and $U_2$)

Two kinds of bloods reacted with the original U reagent, as previously reported (FERGUSON, STORMONT, and IRWIN 1942). More extensive analyses not only of the original U reagent but of others from iso-immune antisera have repeatedly confirmed these results. The reactive bloods which absorbed all the antibodies from these U reagents prepared from iso-immune antisera are designated $U_1$, while those reactive bloods which failed to exhaust all the antibodies are called $U_2$. From the antiserum of each of four rabbits immunized with the cells of cow 63 (CGIJU$_1$WXZ), powerful anti-$U_1$ reagents (titer$>$1:512) were prepared. There was no evidence in these four antisera for U antibodies that reacted with bloods of subtype $U_2$.

By the use of iso-immune U reagents and $U_1$ antibodies from rabbit antisera, three groups of bovine bloods are distinguished: group $U_1$ (reactive with both U and $U_1$ reagents), group $U_2$ (reactive only with iso-immune U reagent) and the group composed of individuals whose cells are non-reactive.

### Subtypes of X ($X_1$ and $X_2$)

As previously stated, the X reagent was prepared from an antiserum produced by transfusing cow 15E with the blood of 63F. From each of two different antisera produced by re-immunizing 15E (BCEFGJMNOYZA′C′-D′E′F′H′) with the bloods of cows 20E (BCEFGJKXD′E′G′H′) and 20Z (ABCEFGJMNXYA′C′E′F′H′), respectively, very similar reagents were prepared which reacted not only with the cells of all individuals of blood type X but with many others as well. When these reagents were absorbed by reactive bloods, of a kind, which were non-reactive with the original X reagent, the antibodies for this class of cells were exhausted and the resulting fractions now paralleled the reactions of the original X reagent. One of these reagents prepared from the anti-20Z serum was absorbed with the bloods of each of 16 cattle of type X and in each instance all the antibodies were exhausted for the bloods lacking X although minor, but, for the present, negligible differences among these 16 absorbing bloods were noted. Accordingly, those bloods which reacted with both the original X reagent and these complex X reagents are

designated $X_1$, while those which reacted only with the more complex X reagents (i.e., the reagents prepared from the anti-20E and 20Z sera) are called $X_2$.

### Subtypes of O ($O_1$, $O_2$ and $O_3$)

Reagents prepared from each of two antisera produced in cow 20E (BCEFGJK$X_1$D′E′G′H′) following transfusions with the blood of 15E (BCEFGJMNOYZA′C′D′E′F′H′) and 20Z (ABCEFGJMN$X_1$YA′C′E′F′-H′), respectively, gave parallel reactions in the differentiation of three serological subtypes of the factor O. The former antiserum was the original source of antibodies for the detection of antigen O (FERGUSON, STORMONT, and IRWIN 1942). Numerous tests following absorptions of these two sera indicated that there was a major fraction of antibodies which could not be obtained separately, by absorption, from the antibodies that reacted with bloods of type O. A complex fraction containing these antibodies in addition to those reactive with bloods of type O was prepared from the second serum. Further attempts were made to absorb the O antibodies without exhausting all the antibodies from this complex reagent (the individual bloods of 15 different cattle of type O were used in one of these tests) but with no success. Bloods of type O absorbed all or most all of these antibodies. Tests following absorptions of this reagent with various reactive bloods demonstrated that there were at least four distinguishable kinds of blood, as shown by the data in table 4. Those bloods designated subtype $O_1$ (previously called O), as already mentioned,

TABLE 4

*Subtypes of factor O*

| SUBTYPES OF O | REACTIONS WITH THE COMPLEX REAGENT | REACTIONS FOLLOWING ABSORPTION OF THE COMPLEX REAGENT WITH | | |
|---|---|---|---|---|
| | | $O_1$ CELLS | $O_2$ CELLS | $O_3$ CELLS |
| $O_1$* | +++ | 0 | + | ++ |
| $O_2$ | ++ | 0 | 0 | + |
| $O_3$ | + | 0 | 0 | 0 |
| —** | 0 | 0 | 0 | 0 |

* $O_1$ is the type previously called O (FERGUSON, STORMONT and IRWIN 1942).

** The non-reactive class of bloods. (+++ strong reaction; ++ moderate reaction; + weak to moderate reaction.)

absorbed all the antibodies. Those bloods designated subtype $O_2$ exhausted all the antibodies except a fraction reactive with $O_1$ cells. Those called subtype $O_3$ absorbed only the antibodies for this subtype and left a fraction which was reactive with bloods of both subtypes $O_1$ and $O_2$.

### Subtypes of E′ ($E_1$′, $E_2$′ and $E_3$′)

Reagents were isolated from each of two rabbit antisera against the cells of cow 8g (AFG$X_2$YE′F′H′) which paralleled the reactions of the E′ reagents

obtained from iso-immune antisera. These E′-like reagents from the two rabbit antisera differed from those that had been found in cattle iso-immune sera in that two kinds of reactive bloods could be distinguished by the kinetics of the reactions. That is, most E′ bloods were rapidly and completely hemolysed (usually within one-half hour) in reagent-dilutions of 1:64 or 1:128, whereas a minority rarely showed more than a trace of hemolysis at one-half hour and usually were only partially hemolysed at the end of two hours. It was possible to exhaust the antibodies for this second class without completely exhausting those for the strongly reactive bloods, although an excess of these weakly reactive cells would absorb all the demonstrable antibodies. The strongly reactive bloods readily exhausted all the E′ antibodies. Accordingly, by the use of these rabbit anti-E′ reagents, two subdivisions of factor E′ were made. The strongly reactive bloods are designated $E_1'$, while the weakly reactive bloods are called $E_2'$.

Results following further absorptions of an antiserum produced in cow 28C by immunizing with the blood of 13C (FERGUSON, STORMONT, and IRWIN 1942) indicated that this serum contained antibodies which appeared to be closely related to the anti-E′ fraction described in that report; that is, it was not possible to exhaust the E′ antibodies without exhausting this particular fraction. A complex reagent containing this fraction in addition to the antibodies for factor E′ was prepared for use in blood-typing. Bloods that reacted with this complex reagent called the $E_3'$ reagent, other than those of subtypes $E_1'$ and $E_2'$, are designated $E_3'$. As in the example of the reagents for the classification of subtypes of factor O, the E′ reagents from the rabbit antisera against the blood of cow 8g and the $E_3'$ reagent from the anti-13C serum differentiated four kinds of cattle blood (i.e. types $E_1'$, $E_2'$, $E_3'$ and those bloods which were non-reactive with these E′ reagents).

## The serological relationship of the antigenic factors B, G and K

The antiserum produced in cow 7C by immunizing with the blood of 20C served as the original source of antibodies for antigens K and A′ (FERGUSON, STORMONT and IRWIN 1942). The absorptions of this serum had indicated that it also contained antibodies for factor B. To obtain from this serum a reagent for the B factor, it would have been necessary to absorb with blood

TABLE 5

*The B-G-K relationship*

| BLOOD-TYPES | NUMBER OF CATTLE |
|---|---|
| —* | 1,579 |
| B | 1,202 |
| G | 1,096 |
| BG | 535 |
| BGK | 1,839 |

* Animals lacking B, G and K.

containing factors K and A′ but lacking B. However, animals possessing K and lacking B were not found. Accordingly, it would appear that bloods of the types BK and B bear the same relationship to each other as do, for example, those of subtypes $T_1$ and $T_2$. It was also observed that the antigenic factor G bears a relationship to K which parallels that of the B factor. The distribution of the known B-G-K blood-types in 6,251 cattle (table 5) illustrates the complete association of the K factor with both B and G even though B and G occurred separately, as well as together, in the absence of K. Consequently, there has been no way of relegating these relationships to a simple system of abbreviated subtypes without retention of the factors B, G and K.

TABLE 6

*Cross reactions of the ($SU_2$) reagent*

| GROUPS | NO. OF CATTLE | REACTIONS WITH REAGENTS | | | |
|---|---|---|---|---|---|
| | | $U_1$ | U | S | ($SU_2$) |
| —* | 637 | 0 | 0 | 0 | 0 |
| S | 675 | 0 | 0 | + | + |
| $U_2$ | 46 | 0 | + | 0 | + |
| $U_1$ | 38 | + | + | 0 | 0 |
| $SU_2$ | 14 | 0 | + | + | + |
| $SU_1$ | 9 | + | + | + | + |

* The non-reactive group.

## The relationship of factor S with the subtypes of U

A reagent prepared from an antiserum produced in 6g ($FNO_1VX_2ZA'D'-E_3'G'H'$) by immunizing with the blood of 26Z ($ABCFHO_1RVX_2ZE_3'H'$) appeared to react with all bloods possessing factor S and a few bloods that lacked S. The cells of the donor were non-reactive with this reagent, and furthermore, lacked the S factor. However, approximately two years prior to the production of this antiserum, the recipient (6g) had produced an antiserum against the blood of cow 33g ($FGJNO_1SYA'E_1'F'H'$) and this serum contained antibodies for S. Furthermore, antibodies for factor S were still demonstrable in a sample of serum taken from 6g just prior to the immunization with the blood of 26Z. It may be assumed, therefore, that the S or S-like antibodies in this second antiserum resulted from the previous transfusions with the blood of 33g.

A comparison of the antigenic formulas of the cattle whose cells reacted with the reagent prepared from the anti-26Z serum demonstrated that the few bloods lacking factor S were all of subtype $U_2$. In accordance with previous experience, it was expected that absorption of this reagent with cells lacking S but possessing $U_2$, and the converse, would sub-divide this reagent into a fraction specific for S and another specific for $U_2$. On the contrary, attempts to fractionate the reagent were not successful, since bloods possessing either

S or $U_2$ exhausted all the antibodies. (The reagent was absorbed with each of seven bloods of subtype $U_2$ and three of type S.) Accordingly, the reagent was designated anti-($SU_2$), the parentheses indicating that the antibodies were not known to be fractionable.

Tests with the S, U, $U_1$ and ($SU_2$) reagents on the bloods of 1,519 cattle are summarized (table 6). The six groups encountered are defined by their reactions with the S, U and $U_1$ reagents. The ($SU_2$) reagent, nevertheless, is potentially useful in that it would differentiate rare bloods containing both the subtype factors $U_1$ and $U_2$ from those possessing factor $U_1$ alone. That is, rare bloods containing both $U_1$ and $U_2$ would be expected to react with the ($SU_2$) reagent, while those possessing the $U_1$ factor alone are not reactive with ($SU_2$) reagent.

The ($SU_2$) reagent, which reacted with the cells of those cattle that possessed either or both of the factors S and $U_2$, did not parallel the reactions of any one of the other reagents. Furthermore, absorptions failed to fractionate the antibodies in this reagent. These are the two criteria which were taken as presumptive evidence, in the earlier studies, for antibodies that are reactive with an antigen distinct from each of the others. Obviously, such a hypothetical unit-antigen must be completely associated, or very nearly so, with each of the factors S and $U_2$. Now it is perhaps more reasonable to assume, that not one but at least two cellular substances were reactive with the ($SU_2$) reagent, as the correlation of the reactions with the presence of either or both the factors S and $U_2$ would indicate. These results, along with those of the subtypes, are considered indicative of overlapping relationships (cross reactions) among the serologically reactive substances of cattle erythrocytes, as will be discussed later.

### *The Inheritance of the Serological Subtypes of T, U, X, O and E′*

Data on the inheritance of factors T, U, X, O and E′ were presented previously (FERGUSON, STORMONT, and IRWIN 1942). The inheritance of the different subtypes is shown in table 7. For example, there are six possible kinds of matings involving the subtypes of T. All 7 offspring from the matings in which both parents were of subtype $T_1$ (i.e., $T_1 \times T_1$ matings) possessed $T_1$. There were no matings of $T_1 \times T_2$. From 63 matings of $T_1 \times —$, there were 39 offspring of subtype $T_1$ and 31 that lacked both $T_1$ and $T_2$. Actually, some progeny with factor $T_2$ are to be expected from matings of the types $T_1 \times T_1$ and $T_1 \times —$. As already mentioned, however, the $T_2$ factor has been observed only in Holstein-Friesians. (In this breed the incidence of $T_1$ was less than one percent while $T_2$ occurred in slightly over four percent of the individuals tested.) As a consequence, there were probably no animals in this study that possessed both of the subtypes of T. There were no matings of $T_2 \times T_2$ individuals among the families that were sampled, but from 42 matings of $T_2 \times —$, there were 21 offspring with $T_2$ as contrasted with 23 offspring that lacked $T_2$. As expected, there were no offspring of either subtype of T among 357 individuals from matings in which the cells of both parents were non-reactive

TABLE 7

*Inheritance of subtypes of T, U, X, O and E'*

| SUBTYPES OF FACTOR | TYPE OF MATING | NUMBER OF OFFSPRING IN THE CLASSES INDICATED | | | |
|---|---|---|---|---|---|
| T | | | $T_1$ | $T_2$ | —* |
| | $T_1 \times T_1$ | | 7 | 0 | 0 |
| | $T_1 \times T_2$ | | 0 | 0 | 0 |
| | $T_1 \times$ —* | | 39 | 0 | 31 |
| | $T_2 \times T_2$ | | 0 | 0 | 0 |
| | $T_2 \times$ — | | 0 | 21 | 23 |
| | — × — | | 0 | 0 | 357 |
| U | | | $U_1$ | $U_2$ | —* |
| | $U_1 \times U_1$ | | 2 | 0 | 1 |
| | $U_1 \times U_2$ | | 0 | 0 | 0 |
| | $U_1 \times$ — | | 17 | 0 | 18 |
| | $U_2 \times U_2$ | | 0 | 0 | 0 |
| | $U_2 \times$ — | | 0 | 19 | 19 |
| | — × — | | 0 | 0 | 397 |
| X | | | $X_1$ | $X_2$ | —* |
| | $X_1 \times X_1$ | | 20 | 3 | 0 |
| | $X_1 \times X_2$ | | 22 | 19 | 0 |
| | $X_1 \times$ — | | 32 | 22 | 6 |
| | $X_2 \times X_2$ | | 0 | 55 | 6 |
| | $X_2 \times$ — | | 0 | 59 | 48 |
| | — × — | | 0 | 0 | 70 |
| O | | $O_1$ | $O_2$ | $O_3$ | —* |
| | $O_1 \times O_1$ | 55 | 2 | 7 | 4 |
| | $O_1 \times O_2$ | 13 | 3 | 2 | 0 |
| | $O_1 \times O_3$ | 32 | 0 | 20 | 7 |
| | $O_1 \times$ — | 61 | 2 | 18 | 14 |
| | $O_2 \times O_2$ | 0 | 0 | 1 | 0 |
| | $O_2 \times O_3$ | 0 | 5 | 9 | 2 |
| | $O_2 \times$ — | 0 | 11 | 9 | 6 |
| | $O_3 \times O_3$ | 0 | 0 | 24 | 4 |
| | $O_3 \times$ — | 0 | 3? | 28 | 22 |
| | — × — | 0 | 0 | 1? | 51 |
| E' | | $E_1'$ | $E_2'$ | $E_3'$ | —* |
| | $E_1' \times E_1'$ | 62 | 9 | 3 | 3 |
| | $E_1' \times E_2'$ | 6 | 5 | 4 | 1 |
| | $E_1' \times E_3'$ | 42 | 1 | 16 | 15 |
| | $E_1' \times$ — | 29 | 0 | 3 | 21 |
| | $E_2' \times E_2'$ | 0 | 2 | 1 | 0 |
| | $E_2' \times E_3'$ | 0 | 3 | 6 | 0 |
| | $E_2' \times$ — | 0 | 2 | 1 | 3 |
| | $E_3' \times E_3'$ | 0 | 0 | 55 | 14 |
| | $E_3' \times$ — | 0 | 0 | 59 | 30 |
| | — × — | 0 | 0 | 0 | 34 |

* The dash (—) symbolizes the absence of the factor.

with the T reagents. Each subtype appears to be inherited as a dominant when contrasted with its absence.

The rare subtype-factors $U_1$ and $U_2$ (see OWEN, STORMONT, and IRWIN 1947 for the frequency of U in two breeds of dairy cattle), like the subtypes of T, most probably did not occur in combination in any of the parental animals of this study since progeny of the subtype $U_2$ were not obtained from the few matings $U_1 \times U_1$ and $U_1 \times$— (table 7). Furthermore, all bloods of subtype $U_1$ tested to date have been non-reactive with the ($SU_2$) reagent, as already pointed out. The data of table 7 indicate that both subtypes of U are inherited as dominants in contrast with their absence.

TABLE 8

*The segregation of subtypes $X_1$ and $X_2$, and $O_2$ and $O_3$ in the progeny of one bull**

| KIND OF MATING | NUMBER OF OFFSPRING WITH | | |
|---|---|---|---|
| | $X_1$ | $X_2$ | —** |
| $X_1 \times$—** | 20 | 19 | 0 |
| $X_1 \times X_2$ | 6 | 3 | 0 |
| $X_1 \times X_1$ | 2 | 1 | 0 |
| Totals | 28 | 23 | 0 |

| | NUMBER OF OFFSPRING WITH | | | |
|---|---|---|---|---|
| | $O_1$ | $O_2$ | $O_3$ | —** |
| $O_2 \times$—** | 0 | 6 | 6 | 0 |
| $O_2 \times O_3$ | 0 | 5 | 8 | 0 |
| $O_2 \times O_2$ | 0 | 3 | 0 | 0 |
| $O_2 \times O_1$ | 11 | 3 | 4 | 0 |
| Totals | 11 | 17 | 18 | 0 |

* Not all the progeny subtyped for X were subtyped for factor O, hence the difference in the two totals.

** The dash (—) symbolizes the absence of the respective factors X and O.

From the matings $X_2 \times X_2$ and $X_2 \times$— there only were two kinds of progeny, *i.e.*, those of subtype $X_2$ and those without the X factor. On the other hand from the matings of $X_1 \times X_1$, and $X_1 \times$— there resulted three kinds of offspring, *i.e.*, those of both subtypes $X_1$ and $X_2$ and those lacking these two factors. Obviously, some of the parents of subtype $X_1$ also transmitted $X_2$, but the recognition of subtype $X_2$ is not yet possible in bloods with factor $X_1$.

Among the sires whose progeny were tested, there was one of subtype $X_1$ that transmitted both subtypes $X_1$ and $X_2$. The results are shown in table 8. In matings to dams lacking both $X_1$ and $X_2$ there were 20 progeny with $X_1$ and 19 with $X_2$. Further, there were no progeny among the total of 51 that lacked both $X_1$ and $X_2$. The results are consistent with the interpretation that the factors $X_1$ and $X_2$ are controlled by allelic genes.

Likewise, offspring of subtypes $O_2$ and $O_3$ resulted from matings of $O_1 \times O_1$

and $O_1$×—, and offspring of subtype $O_3$ resulted from matings of $O_2$×$O_2$ and $O_2$×— (table 7).

The sire that transmitted $X_1$ and $X_2$ as alternatives, also possessed the subtype factor $O_2$. However, he appeared to be heterozygous for a pair of alleles controlling the serological subtypes $O_2$ and $O_3$, as is also shown in table 8.

TABLE 9

*The inheritance of the subtypes $O_1$ and $O_3$ as alternatives*

| KIND OF MATING | NUMBER OF OFFSPRING WITH | | | |
|---|---|---|---|---|
| | $O_1$ | $O_2$ | $O_3$ | —* |
| $O_1$×—* | 14 | 0 | 19 | 0 |
| $O_1$×$O_3$ | 15 | 0 | 10 | 0 |
| $O_1$×$O_2$ | 11 | 1 | 3 | 0 |
| $O_1$×$O_1$ | 39 | 1 | 13 | 0 |
| Totals | 79 | 2 | 45 | 0 |

* The non-reactive group.

There were six sires of subtype $O_1$ that produced progeny of subtypes $O_1$ and $O_3$ when mated to dams lacking these factors. A summary of the matings is shown in table 9. Apparently, these sires were heterozygous for alleles controlling factors $O_1$ and $O_3$.

Similarly, two sires of subtype $O_1$ transmitted $O_1$ and $O_2$ as alternatives. The data are summarized in table 10.

TABLE 10

*The inheritance of the subtypes $O_1$ and $O_2$ as alternatives*

| KIND OF MATING | NUMBER OF PROGENY WITH | | | |
|---|---|---|---|---|
| | $O_1$ | $O_2$ | $O_3$ | —* |
| $O_1$×—* | 8 | 2 | 0 | 0 |
| $O_1$×$O_3$ | 6 | 2 | 0 | 0 |
| $O_1$×$O_2$ | 3 | 6 | 0 | 0 |
| $O_1$×$O_1$ | 22 | 5 | 0 | 0 |
| Totals | 39 | 15 | 0 | 0 |

* The non-reactive group.

The inheritance of the subtypes of factor $E_1'$ parallels that of the subtypes of O.

With the exception of the occurrence of three offspring of subtype $O_2$ from $O_3$×— and one offspring of subtype $O_3$ from —×— matings (table 7), the general rule appears to be applicable, that each of these subtypes of T, U, X, O and E′ is inherited as a dominant when contrasted with its absence. Furthermore, individual animals transmitted the subtypes of X and O as if each of these systems is controlled by multiple alleles. The four exceptions (table 7) are

probably best explained by errors in serological techniques. Unfortunately, it was not possible to re-check the blood types of the parents of these animals.

## PROPOSED CHANGES IN NOMENCLATURE

### Subtypes of C ($C_1$ and $C_2$)

As previously stated (FERGUSON 1941), only three of four expected classes of blood were noted in respect to the reactions obtained with antibodies which detect the antigenic components C and E. These were CE, C and the bloods lacking both factors. Additional absorptions of the original anti-CE reagent and others similar to it have confirmed these observations. That is, bloods of type CE exhaust all or nearly all the antibodies in anti-CE reagents, while those of type C absorb the antibodies for these cells and leave a fraction that is reactive with cells of type CE. These results appeared to be quite analogous to those on the subtypes of T, U and X. To conform with this proposed system of nomenclature, the factor E is no longer designated. Bloods of the former types CE and C are now called $C_1$ and $C_2$, respectively.

### Subtypes of Y ($Y_1$ and $Y_2$)

The relationship between blood types YC′ and Y parallels that between $C_1$ (CE) and $C_2$ (C). In other words, the factor C′ has not been observed independently of Y. Accordingly, bloods formerly designated YC′ and Y are now called $Y_1$ and $Y_2$, respectively.

## DISCUSSION

The experiments described in this and previous reports (FERGUSON 1941; FERGUSON, STORMONT, and IRWIN 1942) have established that the antigenic pattern of bovine erythrocytes is extremely complex and that these serological properties of the cells are gene-controlled. Additional unpublished data indicate that the kinds of antibodies that detect individual differences in bovine bloods may be more numerous than those already described. It has become apparent in the course of these studies, however, that many of the fractions of antibodies do not have reactive patterns that are markedly different from others. As noted in this report, the reactions of the so-called ($SU_2$) reagent were correlated with the presence of either or both the factors S and $U_2$, a fact which eliminated the necessity of proposing an additional antigenic factor, even though this reagent met the requirements, in other respects, of one presumably reactive with a previously undetected antigenic component. Likewise, the innovation of serological subtypes eliminated the use of additional blood factors without sacrificing clarity in conveying the idea of serological differences.

The interpretation of serological reactions in the absence of information concerning the nature of the cellular substances that underlie these reactions is an open question. There are those who have held that antibodies are absolute in their specificities. Consequently, for each demonstrable kind of antibodies there is but one corresponding antigen. As measured in chemical terms, however, the specificities of antibodies are only relative (LANDSTEINER 1945). It is definitely established that antibodies often cross react with substances

related to the homologous antigen, and that the same antigen may react with different antibodies. LANDSTEINER and VAN DER SCHEER (1936) demonstrated that the separation from an immune serum of antibodies different in specificity is no conclusive proof for the existence of several substances or special groups in the immunizing antigen.

It is reasonable to assume, therefore, that some of the results of these serological studies have been consequent on cross reactions or, in other words, overlapping relationships among the serologically reactive substances of cattle erythrocytes. The serological subtypes, the reactions of the so-called ($SU_2$) reagent, the B-G-K relationship, as well as numerous additional associations among and between many of the antigenic factors can most readily be understood on this principle.

While the antigenic factors described in this and previous reports (*loc. cit.*) would appear to be quite definite serological entities, it should be kept in mind that evidence justifying a conclusion that any one of these factors is representative of a single antigenic substance has not been brought forth. On the contrary, the fractionation of numerous reagents, leading to the development of serological subtypes in certain instances, is clearly indicative of minor differences in the reactive substances of different bloods. It should also be emphasized that the demonstration, by absorption, of minor differences in the reactive bloods when tested with the same antibodies (reagent) is not always a necessary index to cross reactions, as the results with the ($SU_2$) reagent would indicate. It is reasonable to believe, therefore, that many of these blood factors may represent only the partial serological properties of more fundamental groups. The identification of substances or groups with overlapping serological properties would depend, in part, upon their distinctive cross reactive properties when tested with a number of different reagents.

The transition from apparently simple cross reactions, the serological subtypes, to those presumably indicative of more fundamental groups may be illustrated by the B-G-K relationship. As will be recalled, the K factor has occurred only in bloods possessing both the factors B and G, even though B and G occur either singly or together in the absence of K. As pointed out elsewhere (STORMONT, IRWIN, and OWEN 1945), certain cattle transmit factors B and G as a unit. Accordingly, a minimum of four reactive groups, symbolized $B_B$, $B_G$, $B_{BG}$ and $B_{BGK}$, could explain the distribution of these three factors. The subscripts of factor B indicate only the partial serological properties of these hypothetical groups when tested with the B, G and K reagents.

Other considerations suggestive of cross reactions were the marked reductions in the titers of the antibodies remaining following absorptions with bloods of subtypes designated by the subscripts 2 or 3. Excessive absorption with such bloods, as noted in the absorption of E′ reagent from rabbit antisera with bloods of subtype $E_2'$, occasionally depleted all the antibodies of subtyping-reagents. Analogous situations in other species may be cited, as the absorption of both alpha and $alpha_1$ antibodies by excessive amounts of cells from human beings of subtype $A_2$, according to WIENER (1943).

In lieu of immunochemical tests, studies of the inheritance of these bovine

blood factors, when considered collectively, should differentiate between most reactions due to overlapping relationships and those due to serologically unrelated substances. Considering the more or less direct relationships that have been proposed between most antigens and their controlling genes (HALDANE 1938; IRWIN and CUMLEY 1943), it is reasonable to assume that substances closely related antigenically and thereby presumably chemically would be controlled by genes closely related in descent, such as alleles and duplicates. For example, the data on the inheritance of subtypes of each of the factors O and X are consistent with the interpretation that cross reactive substances underlying these reactions are controlled by allelic genes. In man, the group specific substances of the A-B-O blood groups are examples of serologically, chemically and genetically related substances (see WIENER 1943).

Ruling out the possibility of cross reactions, arbitrarily or otherwise, the association of antigenic factors in degrees greater than are expected on the basis of chance alone, might be explained, in one way, as due to unusually close linkage of the controlling genes. For example, the two previously described antigens C and E have occurred in only two ways, CE together and C alone (FERGUSON 1941). Assuming absolute specificity of the characterizing antibodies, it would appear that gene *E*, controlling antigen E, is linked with gene *C*. Since the association of factor E with C is apparently complete, there is no way of testing this hypothesis by studying the inheritance of these two factors. It follows, *a priori*, that the group CE (subtype $C_1$) is inherited as a unit. Ferguson suggested that antigens C and E together may be controlled by a single gene and that an allele of this gene controls antigen C. Likewise, a similar interpretation, involving multiple alleles, was advanced to explain the B-G-K relationship (STORMONT, IRWIN, and OWEN 1945). While these explanations based on multiple alleles have certain advantages over the hypothesis of linked genes, they meet with some difficulty in the absence of critical evidence for genes that are capable of producing, supposedly, more than one antigenic substance. The interpretation of the association of antigenic factors on the basis of overlapping relationships of more fundamental groups, however, eliminates this objection. A forthcoming report is concerned with the extension of the B-G-K and C-E relationships into two respective genetic systems of blood groups involving between them the majority of the recognized antigenic factors of bovine erythrocytes.

## SUMMARY

1. Evidence for antibodies that detect additional gene-controlled differences in the serological properties of the bovine erythrocyte is presented. The letters used in characterizing these additional antigenic factors are I′, J′, K′, L′, Z′ and F.

2. Evidence suggestive of overlapping serological relationships (cross reactions) among antigens of the erythrocytes of cattle is also presented. In accordance with this evidence, serological subtypes of each of the antigenic factors T (subtypes $T_1$, $T_2$), U ($U_1$, $U_2$), X ($X_1$, $X_2$), O ($O_1$, $O_2$, $O_3$) and E′ ($E_1'$, $E_2'$, $E_3'$) are proposed. The subtype-symbolism is indicative of minor

serological differences in presumably closely related substances when tested with the same reagent (antibodies). In accordance with this system, it is proposed that the previously termed types CE and C should now be called $C_1$ and $C_2$, respectively. Likewise, the types YC′ and Y should be changed to $Y_1$ and $Y_2$.

ACKNOWLEDGMENT

Most of the iso-immune antisera used in this study were prepared by DRS. L. C. FERGUSON, M. R. IRWIN and R. D. OWEN. Considerable technical assistance was also rendered by DR. R. D. OWEN and MR. RICHARD PIERCE. It is, unfortunately, impractical to cite all the numerous private breeders of cattle and workers at state institutions through whose generous cooperation many of the samples of blood were obtained.

LITERATURE CITED

FERGUSON, L. C., 1941 Heritable antigens in the erythrocytes of cattle. J. Immunol. **40**: 213–242.

FERGUSON, L. C., C. STORMONT, and M. R. IRWIN, 1942 On additional antigens in the erythrocytes of cattle. J. Immunol. **44**: 147–164.

HALDANE, J. B. S., 1937 Essay in "Perspectives in Biochemistry" pp. 1–11 (edited by Needham and Green). Cambridge: at the University Press.

IRWIN, M. R. and R. W. CUMLEY, 1943 Immunogenetic studies of species relationships. Amer. Nat. **77**: 211–233.

LANDSTEINER, K., 1945 The specificity of serological reactions. xiv+310 pp. Cambridge, Mass.: Harvard University Press.

LANDSTEINER, K., and P. LEVINE, 1928 On the inheritance of agglutinogens of human blood demonstrable by immune agglutinins. J. exp. Med. **48**: 731–749.

LANDSTEINER, K., and J. VAN DER SCHEER, 1936 On cross reactions of immune sera to azoproteins. J. exp. Med. **63**: 325–339.

OWEN, R. D., C. STORMONT, and M. R. IRWIN, 1947 An immunogenetic analysis of racial differences in dairy cattle. Genetics **32**: 64–73.

STORMONT, C., and R. W. CUMLEY, 1943 Cellular antigens in cattle blood. J. Hered. **34**: 34–41.

STORMONT, C., M. R. IRWIN and R. D. OWEN, 1945 A probable allelic series of genes affecting cellular antigens in cattle. (abstract) Genetics **30**: 25–26.

WIENER, A. S., 1943 Blood groups and transfusion 3rd ed. xix+438 pp. Springfield, Illinois: Charles C Thomas.

# ABSTRACTS OF PAPERS PRESENTED AT THE 1949 MEETINGS OF THE GENETICS SOCIETY OF AMERICA

## NEW YORK CITY, DECEMBER 28 - 30, 1949

ALTENBURG, LUOLIN, EDGAR ALTENBURG, HELEN U. MEYER and H. J. MULLER, The Rice Institute, Houston, Texas: The lack of proportionality of mutations recovered to dosage of ultra-violet administered to the polar cap of Drosophila. — The frequency of lethal mutations induced by "germicidal" ultra-violet (chiefly 2537°A) in the second chromosomes of germ cells of the embryonic polar cap does not increase proportionately with rise in dose. Although at lower doses more nearly proportionate, the frequency appeared, as the dose rose, to approach a maximum of about 20%, beyond which no significant increase was observed. In one series, a 14-fold dose increase did not even double the frequency, 11.5%, found at the lowest dose. An improved genetic technique was employed. Our data comprise a total of over 7,000 treated and 4,000 untreated second chromosomes which had been non-lethal at fertilization. — Changing the angle of the incident ultraviolet was found to have pronounced influence, an eightfold increase in mutation frequency being, in one series, caused by changing the illumination from sidewise to vertical to the cap. The cells must therefore shade one another markedly in sidewise treatment, becoming very unequally exposed. Since those more strongly exposed would be selectively killed or retarded, part of the disproportionality in the frequency-dosage relation would thus be explained. — However, this explanation hardly seems sufficient for our more vertically treated series. Possibly the cells vary in their sensitivities to both the directly detrimental and the mutagenic effects in a correlated way. Another possibility is that higher exposures (chiefly perhaps through light of longer wave-lengths, as found by others in bacteria and viruses), may cause desensitization to, or may block or reverse, mutagenic processes initiated by ultraviolet but not yet completed. (Work supported in part by grants from U. S. Public Health Service, on recommendation of National Advisory Cancer Council).

ATWOOD, K. C., Columbia University, New York, N. Y.: The role of lethal mutation in the killing of Neurospora conidia by ultra-violet light. — Two-component Neurospora heterokaryons produce some heterokaryotic macroconidia and some which are homokaryotic for each component. This situation is adapted to comparing genetic with non-genetic

lethal effects as follows: Macroconidia of a heterokaryon between any biochemical mutant and a morphological mutant, amycelial-422, are treated with a mutagenic agent and plated in minimal sorbose medium. Two colony types appear; colonies of normal morphology arise from conidia which are heterokaryotic, and amycelial colonies arise from conidia homokaryotic for the 422 component. The heterokaryotic colonies are isolated, allowed to conidiate, and the conidia plated in minimal sorbose medium. Those isolates whose conidia produce exclusively normal colonies represent cases where lethal mutation has occurred in the nucleus (or nuclei) of the 422 component within the treated heterokaryotic conidium. Among conidia from such isolates, those homokaryotic for 422 are also homokaryotic for an induced lethal and therefore form no colonies. With 29997 (ornithineless) as the biochemical mutant component this system was used to test the hypothesis that ultraviolet light kills multinucleate conidia by inducing a homologous lethal mutation in each nucleus. This hypothesis predicts that the proportion of surviving conidia carrying lethal mutations should continue to increase with decreasing survival. In experiments with UV the incidence of isolates carrying lethals in their 422 component reached a maximum of 25% at 20% survival and declined thereafter. These data, plus the survival curves, indicate that many conidia killed by high doses of UV are conidia in which no mutations have been induced.

BAKER, WILLIAM K., and ELIZABETH SGOURAKIS, Oak Ridge National Laboratory, Oak Ridge, Tenn., and Department of Zoology, University of Tennessee, Knoxville, Tenn.: Alteration of the X-ray sensitivity of Drosophila by means of respiratory inhibitors. — Males of the Oregon-R strain of D. melanogaster were subjected to X radiation (1000, 3000, or 5000 r units, 250 kvp, 15 ma) while in an atmosphere of either $0_2$ or $N_2$ at an approximate temperature of either 27° or 2° C. The number of X-chromosomes which bear recessive lethal mutations was determined by mating the treated males to the females of Muller-5 stock using the technique outlined by Spencer and Stern (Genetics 33: 43-74, 1948). Depending upon the dosage, a 40-70 per cent reduction in the number of sex-linked lethal mutations is observed in the sperm from the flies irradiated in $N_2$ as compared to those treated in $0_2$. Indirect evidence also indicates that the induced frequency of dominant lethals is greatly reduced when the flies are X-rayed in $N_2$. A significantly greater number of mutations is induced in the flies treated in $0_2$ at the cold temperature than in those maintained in $0_2$ at the warm temperature. This "temperature effect" is not evident when the flies are irradiated in $N_2$ thus indicating that this effect is related to the $0_2$ concentration in the sperm. The data are compatible with the differences in solubility of $0_2$ at the temperatures used. Control experiments were performed in which series of flies were subjected to each gas at the two temperatures without irradiation. These experiments show no alteration of the spontaneous mutation rate.

BOOTHROYD, E. ROGER, McGill University, Montreal, Quebec, Canada: Differential contraction of the chromosomes of Trillium in relation to the division cycle. — The chromosomes of certain plants and animals, when kept at low temperatures, show specific regions of reduced diameter and staining capacity at metaphase and anaphase. In an earlier paper it was shown that these regions were under-contracted compared with the rest of the chromosomes. In the present study the relationship of differentiation to the division cycle was investigated. Pollen grains of Trillium erectum, germinated at room temperature, were refrigerated at 3°C and 6°C. At 3°C mitosis was arrested. Refrigeration at 6°C was started when most nuclei were in early prophase and a few in mid or late prophase. After 142 hours some divisions had reached metaphase. These showed clear-cut differentiation similar to that in root tip chromosomes after four days cold, at which time maximum differentiation is reached. Pollen tubes in which mitosis was arrested at metaphase by 3°C refrigeration showed only very slight traces of differentiation. It is concluded that, contrary to the assertions of Darlington and LaCour, differentiation can be produced by cold treatment started at mid or late prophase. The fact that there is a differential contraction, probably with some parts remaining partially contracted while others continue to contract to metaphase, may explain why very little differentiation was seen at the lower temperature. A more detailed study of differentiation and the division cycle is planned.

BOYES, J. W., McGill University, Montreal, Quebec, Canada: Somatic chromosomes of parasitic diptera. — The somatic chromosomes in the sub-oesophogeal ganglia in genera of the Sarcophagidae and Tachinidae families of Diptera have been studied in a joint project with the Dominion Parasite Laboratory at Belleville, Ontario, Canada. Aceto-carmine squash preparations have been used and representative species of over a dozen different genera have been studied. There are usually six pairs of chromosomes which can be distinguished morphologically. The chromosomes are subtelocentric to nearly metacentric and by analysis of (a) the relative lengths of their arms, (b) the percentage that each pair represents of the total length of the complement, (c) the ratios of x:y and (d) the location of secondary constrictions, it has been possible, so far, to distinguish the genomes of different genera. Salivary chromosomes in this group are less suitable for analysis than in many other Diptera.

BRAUN, WERNER and KEITH H. LEWIS, Camp Detrick, Frederick, Md.: Colony morphology of E. coli mutants as a tool for genetic studies. — With the help of obliquely transmitted light and special solid media slight differences in colony morphology, otherwise unrecognizable, become apparent. Examination of 36 mutant cultures of E. coli, differing in biochemical requirements or resistance to various agents showed that practically all these mutants differed in colony-morphology from that of

their parent type; most of those tested also differed from each other. Several colony-types could be detected in some supposedly pure mutant cultures. Each of these types could be picked and gave rise to subcultures with uniform colony-types. Such subcultures differed from each other in growth-rates even though they appeared identical for the mutant characteristic (e.g., streptomycin-dependence) for which they had been selected. The value of using colony-morphology as a simple indicator of a population's homogeneity is thus indicated. Differences in colony-morphology also permit a simple analysis of interactions between mutant populations. Thus in mixed cultures of streptomycin-dependent (sd) and streptomycin-resistant (sr) mutants it was observed that the presence of sr mutants may inhibit the establishment of sd mutants, presumably due to the destruction or utilization of streptomycin by sr mutants. Aging on McConkey agar has been found to induce (or select?) a great number of mutants in certain cultures. One of the mutants thus obtained proved to be an unstable type which constantly gave rise to two types, segregating at the ratio of 2:1. Further testing of this unstable type produced preliminary data which suggest that this constant segregation may be due to the presence of a lethal factor in a heterozygous diploid.

BRAVER, GERALD, and JERRY L. BLOUNT, University of Missouri, Columbia, Mo.: Somatic eliminations of ring chromosomes in Drosophila melanogaster. — The stable $X^{c2}$ and unstable $w^{vc}$ ring chromosomes of D. melanogaster have been studied for the detection of somatic elimination of the rings. The per cent of gynandromorphs among $X^{c2}$/dl-49, y Hw $m^2 g^4 f^5$ females is approximately 0.68 and in $w^{vc}$/dl-49 females is approximately 5.0. Aceto-orcein smears of larval neuroblast cells from similar females showed bridges in 12 per cent of the $X^{c2}$ anaphases and in 22 per cent of the $w^{vc}$ anaphases. The bridges consist of interlocked rings in most cases and of unmistakable long dicentric rings in occasional cells. No anaphases were found containing bridges of non-ring dicentric type, which would be expected in the case of crossing over between the ring and dl-49 chromosomes. If sister strand exchange were responsible for bridge formation, a high frequency of single-exchange dicentric rings would be expected; the rarity of such dicentrics favors the conclusion that eliminations result principally from errors in ring chromosome synthesis.

BROWN, SPENCER W., University of California, Berkeley, Calif.: Differentiated chromosomes in several genera of the Solanaceae (preliminary observations). — Chromatic-achromatic differentiation of chromosomes has been found in the single species examined of each of the following genera: Capsicum, Lycopersicon, Nicotiana, Petunia, Salpiglossis, Solanum. Although some species appear to be unfavorable for a detailed cytological analysis, a few of the pachytene chromosomes, or prophases of tapetal divisions could be studied. In general, the chromatic zones

seem to be adjacent to the centromeres while the achromatic zones are distal. Variation among the species examined has been observed in the size of the telochromomeres, chromatic elements which occur at the ends of the chromosome arms, distal to the achromatic zones. Further work is needed to determine whether the chromosomes of this family are in general characterized by a differentiated morphology.

BURDETTE, WALTER J., Louisiana State University, School of Medicine, New Orleans, La.: Lethal mutation rate following serial administration of methyl-bis (betachloroethyl)amine hydrochloride. — Drosophila females heterozygous for wild type and $sc^8$ $w^a$ B chromosomes were treated by dropping 2% methyl-bis(betachloroethyl)amine hydrochloride on the abdomen. They were mated with Muller-5 males, and the heterozygous females in the progeny were treated in a similar manner serially for 12 generations. Samples from the population were tested for lethals on the X chromosome each generation. During the first three generations sesame oil was used as a solvent, and during the last nine generations propylene glycol was substituted. A total of 149 lethal mutations were detected among 17,052 chromosomes tested (0.87%), 108 on the wild type and 41 on the $sc^8$ $w^a$ B chromosome. Sixty-seven of the lethals on the wild type chromosome were obtained from 8 parents each of which presumably carried over a lethal from the previous generation since all offspring tested carried lethals. It was possible to trace 3 of these 8 to a common origin. In addition 7 parents accounted for 21 lethals on the wild type chromosome out of 162 matings, and 4 parents accounted for 13 lethals on the $sc^8$ $w^a$ B chromosome out of 131 matings. In each instance more than one lethal was present in each group with a common origin which may be explained by a mosaic effect due to the chemical administered. Control studies showed 2 lethal mutations in 2822 chromosomes tested in heterozygous females (0.079%). (Work aided by a grant from The National Cancer Institute, U. S. Public Health Service).

BURNHAM, C. R., University of Minnesota, St. Paul, Minn.: Chromosome segregation in chain-forming translocations in maize. — In three chain-forming translocations (one break in the satellite), less than 0.5 per cent of adjacent 2 segregations were observed. Two of these translocations have short interstitial segments and therefore appear to behave differently from the ring-forming translocations of similar type; that is, in a group of eleven ring-forming translocations with short interstitial segments, the frequency of adjacent 2 segregation (non-disjunction of the segments carrying homologous centromeres) was relatively high (avg. of 25.0 per cent). The other chain-forming translocation has a long interstitial segment and its behavior agrees with ring-forming translocations of similar type. In chains, there is a low frequency of adjacent 2 segregation whether crossing over in the interstitial segment is low or high,

whereas in rings a low frequency of crossing over is correlated with a relatively high frequency of adjacent 2 segregation.

CARSON, HAMPTON L. and HARRISON D. STALKER, Washington University, St. Louis, Mo.: Natural breeding sites for Drosophila robusta. — This species, a characteristic inhabitant of the deciduous forest of the eastern United States, has been found breeding on infected sap exudations, or slime fluxes, of 13 species of deciduous trees near St. Louis, Missouri. Exuding of sap is commonly called fluxing; when the bleeding is sustained, the sap may become contaminated with various yeasts, bacteria and fungi, giving rise to the condition known as slime flux. Of the hosts for D. robusta, 9 are indigenous (American Elm, Slippery Elm; Red, White and Pin Oak; Wild Cherry, Red Mulberry, Black Willow and Cottonwood) and 4 introduced (Chinese Elm, Plum, Peach and White Mulberry). Of 110 slime fluxes selected as favorable for the breeding of Drosophila, 27 were positive for D. robusta. All stages of development from eggs to emerging adults were recovered in nature. The suitability of a slime flux for oviposition and larval development appears to vary profoundly with time, within a species of tree and between tree species. Although D. robusta is the most abundant species breeding on slime fluxes in June and July, when the observations were made, D. melanica, D. victoria, D. athabasca mahican and D. repleta have also been bred from this material. All but the latter are considered to be "native" species and are rarely bred from domestic materials. Knowledge of breeding sites of native species should provide new means of sampling natural populations for genetical or cytological analysis, as well as facilitate interpretation of population cycles and the modus operandi of natural selection.

CASPARI, ERNST, Carnegie Institution of Washington, Cold Spring Harbor, N.Y.: Serological differences between $a^+a^+$ and aa Ephestia. — Rabbits were immunized with saline extracts from $a^+a^+$ and aa Ephestia larvae which had been previously backcrossed to each other for 8-9 generations. The sera were tested by the precipitin reaction against both types of antigen. No differences in the reaction of the sera with homologous and heterologous antigens were found in titrations by serial dilution. Optimum proportions titrations, on the other hand, produced in every serum a higher titer with the homologous than with the heterologous antigen, the titer being expressed in mg antigen nitrogen. Sera absorbed with the heterologous antigen at the optimum proportions point reacted consistently more strongly with the homologous than with the heterologous antigen. One anti $a^+a^+$ serum, after absorption with aa antigen, did not react with aa extracts, but still reacted with $a^+a^+$ antigen. This serum was tested with different $(NH_4)_2SO_4$ fractions from $a^+a^+$ and aa extracts. It reacted only with the euglobulin fraction from $a^+a^+$ extracts. It is concluded that antigenic differences between $a^+a^+$ and aa Ephestia larvae

exist, and that at least one of the differential antigens is in the euglobulin fraction.

CHASE, HERMAN B., Brown University, Providence, R. I.: Inheritance and selection of insulin-resistance in mice. — In a previous paper, the inheritance of insulin tolerance or resistance in mice was demonstrated. With a selection program involving KL (resistant) X C57 Black (normal), it is now apparent that the multiple factor hypothesis suggested at that time is correct. The maximum dose (Iletin, Lilly) survived at 35 days by C57 Blacks is 3 units, by KL's is 300 units; at 70 days by C57 Blacks is 8 units, by KL's is 500 units. At 35 days the lowest dose which has killed a KL is 240 units. The selection consists of testing all $F_2$ offspring with 100 or 200 units at 35 days of age. The survivors are mated; their offspring are tested with the same dose; again the survivors are mated, and so on. A total of 256 animals in this program have been tested thus far. About half of the $F_2$ generation survives 100 units, and this proportion persists in subsequent generations. Twenty-eight per cent of the $F_2$ generation survives 200 units, but the proportion in subsequent generations approaches half. Although 100 units far exceeds normal tolerance under any fasting and feeding conditions used, continued selection at that level is little effective in producing homozygosity. The 200 unit level is more effective, in fact selection with 300 units from this line has proved practicable. Considering the arbitrary cleavages imposed by the doses used, an estimate of three pairs of factors, additive and no dominance, is suggested.

COHEN, SEYMOUR S., and RACHEL ARBOGAST, Children's Hospital of Philadelphia, and University of Pennsylvania, Philadelphia, Pa.: A biochemical study of the mutual reactivation of ultraviolet-inactivated bacteriophages in their host bacteria. — We have tested Luria's hypothesis of independent gene pool synthesis under conditions of mutual reactivation of ultraviolet-inactivated bacteriophages by following various aspects of nucleic acid metabolism. The relations between genetic phenomena and desoxyribose nucleic acid (DNA) in this system will be summarized. In E. coli synthesizing T2r$^+$ virus the constant rate of increase in 2600 Å absorption of the infected culture slightly preceded and was equivalent to the rate of DNA synthesis. DNA synthesis paralleled and preceded virus synthesis. In cells treated with several particles of irradiated T2r$^+$ (hit ca 3.3 times, or about one-eighth the number of genetic units estimated by Luria) DNA synthesis was totally inhibited for about 45 minutes. One intact T2r$^+$ had then been formed within the cell and DNA synthesis began at the rate characteristic of normal infection. Thus there was no independent DNA synthesis referable to the undamaged sites: DNA synthesis required the interdependent activity of all the genetic material present in a complete particle. However, the ultraviolet absorption technique and subsequent chemical analyses showed that vigorous

ribonucleoprotein synthesis occurred before reactivation. The synthesis of ribose nucleic acid (RNA) stopped when an intact virus particle appeared and DNA synthesis began at the former rate of synthesis of RNA. Thus the ultra-violet damaged virus appeared to affect only DNA synthesis, whereas active virus blocked RNA synthesis. It was also shown that small numbers of inactive units inhibited DNA and virus synthesis despite the presence of excess active units.

CONGER, ALAN D., Oak Ridge National Laboratory, Oak Ridge, Tenn.: The effect of boron enrichment on slow neutron irradiated tissues. — The effect of slow neutrons on tissue is an indirect one, and results from the ionizing radiations emitted by atoms which have captured a slow neutron. A previous investigation of the effect of slow neutrons on Tradescantia chromosomes had indicated that 99 per cent of the ionization dose absorbed in tissue was due to only three elements, hydrogen (emitting a gamma ray), nitrogen (proton) and boron (alpha particle). Boron alone was responsible for 32 per cent of the ionization dose absorbed. The considerable importance of boron in these slow neutron exposures was surprising in view of its minute amounts in tissue, only 2.9 p.p.m. of the wet weight in this case, but was due to two factors: (1) the very high probability that a boron atom will capture a neutron, and (2) the fact that the alpha particle emitted loses all its energy within the tissue. The reaction is:

$$B^{10} + \text{neutron} \longrightarrow \left[B^{11}\right] \longrightarrow Li^{7} + \text{alpha particle.}$$

To test and confirm the importance of boron in tissues, inflorescences from Tradescantia plants grown in boric acid solution for about two months were exposed to slow neutrons, and the frequency of chromatid aberrations induced was compared with the frequency from normal non-enriched inflorescences. It was found that, for the same dose of slow neutrons, the boron enriched plants showed from four to five times as many chromatid aberrations as the nonenriched plants, confirming the expectation that boron, in spite of its micro amounts, is one of the most damaging elements when small volumes of tissue are exposed to slow neutrons.

da CUNHA, ANTONIO BRITO, Columbia University, New York, N.Y., and University of Sao Paulo, Brazil: Polymorphism in natural populations of Drosophila. — Natural populations of most Drosophila species are rather uniform in their external traits. At least two species, Drosophila polymorpha and D. montium show however a considerable variability in the coloration of their abdomens. In the former of these species it has been possible to show that the extremely dark and extremely light specimens are homozygous, and intermediate specimens are heterozygous, for two alleles of a single gene. Furthermore, the heterozygotes are adaptively superior to both homozygotes. This is, accordingly, a case of balanced polymorphism. Far more important in the adaptation of species of

Drosophila to their environments is however physiological polymorphism, which is frequently connected with cytologically visible polymorphism in the structures of the chromosomes. The amount of polymorphism shown by a species may vary in different geographic regions. An attempt is made to correlate the amount of polymorphism with characteristics of the environment prevailing in different territories.

DEMPSTER, EVERETT R. and MICHAEL LERNER, University of California, Berkeley Calif.: Heritability of threshold characters. — Quantitative methods have been published for handling all-or-none characters whose appearance or non-appearance depends on a number of gene pair differences as well as environmental variability. These methods are based on specifying the genotype of any individual as the probability that it would show the character when exposed to one environment chosen at random from the whole array of environments. Characters of this type have also been discussed in terms of an hypothetical underlying variate which, when it exceeds a threshold, leads to the appearance of the character. On the simplest model of this kind gene differences have additive effects on the underlying variate and environmental influences are normally distributed. In this model the effects of gene differences expressed on the probability-of-appearance scale are partly non-additive and environmental variance is dependent on the genotypic level. Results of the following calculations are presented for several combinations of parameters in the model described: (1) Proportions of genetic variance that are non-additive; (2) Proportions of non-additive genetic variance included in different kinds of heritability estimates; and (3) Comparisons of gains that would be actually achieved from mass selection with gains calculated from heritability estimates on the probability-of-appearance scale. It appears that expected gains computed on the latter basis will often be in error when the incidence in the population is close to zero or unity.

DERMEN, HAIG, Bureau of Plant Industry, Beltsvile, Md.: The genetics of histogenic type of variegation in tobacco. — A variegated tobacco plant (Nicotiana tabacum) was used for breeding to determine the genetic nature of the histogenic type variegation. For this experiment: a, the variegated plant was selfed; b, the variegated plant was pollinated with the pollen of a normal, non-variegated plant; c, a normal plant was pollinated with pollen of the variegated plant; and d, plants of the c class were selfed. In all these, normal amounts of seeds were obtained, which indicates that both female and male gametes of the variegated plant were fully functional. However, the seedlings of a and b grew to the stage of cotyledon expansion, and then all died. They were of a light green shade as compared with the more intense green color of normal seedlings. Every seedling belonging to class c grew normally into normal green seedlings, some of which were carried to maturity. The selfed d plants

gave normal germination and normal seedlings. These results show that the variegation in this tobacco plant, and most probably in other kinds of plants similarly variegated, is due to a mutation within the cells of only one histogenic layer and affecting the cytoplasm of only that layer. The affected layer in the tobacco is the one that gives rise to the sporogenous tissue of both anther and ovule, and to the colorless margins of the leaves of the variegated plants. The nucleus is not affected and only the egg cells carry the affected cytoplasm, not the pollen grains.

EVERETT, HERBERT L., Connecticut Agricultural Experiment Station, New Haven, Conn.: Isolation of a genic series controlling chloroplast pigment production in Zea mays L. — A maize inbred, in which normal chloroplast formation is associated with a dark yellow endosperm color and albinism is associated with a light endosperm color, has been crossed with several maize inbreds whose chlorophyll phenotypes vary from "very dark" to "very light." Plants of these crosses were selfed and ear progeny which showed endosperm color segregation were raised subsequently in the greenhouse. A majority of the seedling lots showed the pleiotropic effect whereby the plantlets from the light endosperm kernels were all albino. However, a few light endosperm progeny showed 3:1 segregations for normal chloroplast formation and albinism. By selecting the normal green plants, additional genes controlling the formation of normal chloroplast pigments have been isolated. Moreover, at least one of these genes permits completely normal plant growth and maturity in the presence of the homozygous pleiotropic allele for albinism. These major genes for plant pigment formation seem to control relative amounts of the various plastid pigments, and this control of pigment-ratios probably influences the efficiency of the photosynthetic processes as well as the heterotic vigor in maize.

FABERGÉ, A. C., University of Missouri, Columbia, Mo.: Chromosome breakage by x-rays at low temperature and the radiodecomposition of water. — A year ago data were presented at the meeting of this Society, showing that when Tradescantia pollen is X-rayed at liquid air temperature, the sensitivity of the chromosomes (as measured by the number of breaks observed in pollen tube divisions) is about one fifth of that at +25° C. Several intermediate temperatures have now been explored. The sensitivity curve has a dip in the region of 0° C., and thereafter falls gradually. Its general character resembles the curve of $H_2O_2$ production plotted against temperature, when water containing oxygen is X-rayed (Bonet-Maury and LeFort, 1948, Nature 162: 381-382) but differs in that $H_2O_2$ production stops completely below -116° C., whereas chromosome breaks are still produced at -192° C. All previously reported effects of temperature on the X-ray sensitivity of Tradescantia chromosome could be explained in terms of restitution and rejoining. The author believes that the effect now described is not of this kind, and occurs largely or

entirely at the level of the chemical steps which intervene between ionization and chromosome breakage. X-ray action can still usefully be regarded as particulate, and a target theory is still possible, but a factor must be added to take into account the spread of effect around ionization columns.

FANKHAUSER, G. and R. R. HUMPHREY, Princeton University, Princeton, N. J. and University of Buffalo, Buffalo, N. Y.: The progeny of triploid axolotls. — Twenty-two spawnings were obtained from triploid axolotl females mated with diploid males, including a total of 2076 fertile eggs. The chromosome numbers of 445 larvae, as determined from counts in the amputated tailtips, were as follows: 27 chromosomes, 2 larvae; 28 (diploid), 8; 29 to 41, 377; 42 (triploid), 0; hypertriploid, 3; 56± (tetraploid), 48; 70± (pentaploid), 3; 98± (heptaploid), 2; mosaics, 2. The high number of tetraploid offspring indicates frequent failure of one of the meiotic divisions in triploid oocytes. Compared with the theoretical frequencies expected on the basis of random segregation of the third set of chromosomes at meiosis, the actual distribution of chromosome numbers between diploid and triploid showed a shift of the mode from 35 to 33, probably because of the elimination of chromosomes during meiosis rather than differential mortality of young embryos. The viability of the tetroploid larvae was frequently normal, while that of the aneuploid individuals with chromosome numbers between diploid and triploid was much reduced. Altogether, only 35 animals survived for periods ranging from three months to over three years; these included 1 diploid, 10 hyper-diploids (29 to 32 chromosomes), 2 hypo-triploids (38 and 41 chromosomes), and 22 tetraploids or near-tetraploids. Typical symptoms noted in aneuploid, non-viable embryos and larvae were complete lack or subnormal development of circulation with frequent stasis and hemorrhages, associated with ascites and, more rarely, edema, indicating a particularly high sensitivity to chromosomal unbalance of the processes involved in the establishment and maintenance of circulation.

FOGEL, SEYMOUR, Brooklyn College, Brooklyn, N. Y.: A mutable gene at the R Locus in maize. — While surveying the mutational capacities of single alleles at the R locus, four independently occurring mutations, of a type previously unknown for the locus, were detected in progenies from irradiated and non-treated male gametes. The mutants were found as sectorial seedlings. They are essentially similar and derive from a single parental gene $r^{ch}$. In genetic behavior they conform to the hypothesis that these newly derived alleles represent highly mutable genes. The mutable gene, phenotypically equivalent to r8(green plant) can apparently stabilize at any one of three sharply distinguishable levels of effect. Reversions to alleles indistinguishable from the parental $r^{ch}$ gene, along with those of intermediate and null effect have been recovered. Reversion rates to the various levels of effect are widely different. The external

appearance of the sectorial plants, together with genetic transmission data, suggests that both the time and frequency of reverse mutation is under genic control.

FOGEL, SEYMOUR, Brooklyn College, Brooklyn, N. Y.: A reconsideration of the dp locus in Drosophila melanogaster. — The $F_1$ progeny from irradiated wild males (6000r) crossed to Cy pr (2R + 2L) / dp b pr c px sp were examined for visible mutations at the marked loci. The pooled data, involving approximately 25,000 individuals, for the six tested loci, indicate wide differences in the relative susceptibility for mutation in response to X-rays. Further study of the dp mutants shows them to be of widely diverse phenotypic effects. Among the mutants are those which affect the thoracic vortices or the truncation of the wings, either independently or in combination. On the basis of present data, the constellation of phenotypic effects associated with the dp locus may be viewed as due either to separable physiological components of the action of a single gene, or to the action of closely linked reduplicating units.

FROLIK, E. F. and C. R. BURNHAM, Nebraska Agricultural Experiment Station, Lincoln, Neb., and Minnesota Agricultural Experiment Station, St. Paul, Minn.: Chromosome segregation in maize crosses producing rings of six chromosomes. — This study was undertaken to determine the influence of breakage position on chromosome segregation in rings of six chromosomes. Crosses studied included 50 different translocations in various rings of six. Corresponding parental rings of four for the same translocations were also studied. Mean differences in pollen sterility for groups of crosses in comparisons involving various physical factors in the make-up of the rings were small in all cases. This was true for both rings of six and rings of four. These results indicate that if completely directed segregation occurs in maize, it must be of much less common occurrence than in Oenothera and Datura. It is suggested that the most likely means of finding directed segregation in maize is through genetic control.

FULLER, JOHN L., Jackson Memorial Laboratory, Bar Harbor, Maine: Genetic control of audiogenic seizures in hybrids between dba subline 2 and C57 black subline 6. — Susceptibility of the house mouse to audiogenic seizures has been attributed by Witt and Hall to a single dominant gene (As). Their criterion was the occurence of a convulsion in one or more of 4 exposures to a ringing doorbell. Reinvestigation of this problem using a different dba subline and a quantitative grading of susceptibility has shown that all hybrids are intermediate to the parent strains. Convulsion risks in percentage on first exposures were: dba (n=88) 99%; Bx dba (n=191) 92%; $F_1$ (n=100) 32%; $F_2$ (n=107) 58%; B x C57 (n=151) 23%; C57 (n=50) 0%. The risk is reduced on subsequent exposures. Seizure susceptibility and the ability to recover from seizures appear to be

inherited independently. Genes do not control susceptibility as an all or none character, but determine an individual's average position on a physiological scale. Non-genetic factors operating randomly also influence position on the scale and are determinate in individuals genetically near the seizure threshold. A rough calculation of the number of gene pairs involved in the parental difference gives 3 or more. By a combination of breeding and selection techniques, animals with various predictable seizure risks can be produced. The physiological and genetic implications of these findings will be discussed.

GARBER, E. D., University of California, Berkeley, Calif.: Cytotaxonomic studies in the genus Sorghum. — The basic chromosome number of Sorghum is five and is known only in two of the six subgenera: Para-sorghum and Stiposorghum. The following cytological characters furnish the basis for the construction of a key not only to the subgenera but also to the species of Para-sorghum and Stiposorghum: appearance of chromosomes at pachytene and diakinesis, chromosome size at diakinesis, number of nucleolus chromosomes, and chiasma frequency. A cytological analysis of an interspecific hybrid between an autotetraploid species, S. leiocladum (4n=20), and an allotetraploid species, S. nitidum (2n=20), in the subgenus Para-sorghum indicates that one of the two genomes in the latter species is closely related to the genome in S. leiocladum. Although the two genomes in S. nitidum appear to be nonhomologous since only ten bivalents are observed at diakinesis and MI, pairing between chromosomes in different genomes occurs in the interspecific hybrid. The genera Sorghum and Cleistachne constitute a circle of affinity in the subtribe Andropogoninae. The cytotaxonomic data suggest that polyploidy was a major factor in the evolution of species in this subtribe. Cleistachne (x=9), an advanced genus, may have been derived from an ancestral polyploid stock with a somatic chromosome number of 20. An hypothesis synthesized from the combined data of morphology, distribution, and cytology places the probable center of origin of Sorghum in southern Asia.

GARDNER, ELDON J., Utah State Agricultural College, Logan, Utah: Genetic analysis of tumorous head in Drosophila melanogaster. — An abnormality expressed as irregular growths in the head region of Drosophila melanogaster has been shown to be hereditary and is being subjected to a genetic analysis. Two major genes are involved. One is a sex-linked recessive or slightly dominant gene with a maternal effect. The other is semidominant, homozygous viable at 22°C, and is located in the third chromosome. The inbred (tu-h) stock at present has an average penetrance of 76 percent at 22°C. The penetrance is increased progressively with increased temperature from 56 percent at 15°C to 93 percent at 30°C. The maternal effect is influenced by temperature change but the action of the third chromosome gene alone is not altered by temperature change.

Genetic modifiers have been found which influence the action of the third chromosome gene but none have been found to influence the maternal effect. A temperature effective period during the first 24 hours of development, in the egg and early larval stage, has been identified. An average of about 30 percent of abnormal flies is produced in the $F_1$ when tu-h females are crossed with males from laboratory stocks. Less than 1 percent of abnormal flies result from the reciprocal cross. The $F_2$ results are comparable for reciprocal crosses and only small percentages of abnormal flies are obtained. The Oregon R and Stephenville stocks carry sex-linked genes which produce the maternal effect. These stocks lack the third chromosome gene and, therefore, do not express the abnormality.

GECKLER, ROBERT P., Vanderbilt University, Nashville, Tenn.: Inheritance of changes induced by nitrogen mustard in Paramecium aurelia, variety 4. — Chemical induction of mutation by nitrogen mustard was investigated in order to extend previously obtained results in other organisms by other workers to Paramecium aurelia and to investigate the mutability of cytoplasmic factors. Reduction in viability of animals after fertilization was used as a measure of effect. Selected data are presented to show that some of the changes induced in Paramecium aurelia are inherited through nuclear mechanisms and that in some of the changes the cytoplasm is involved. By exposing single animals to nitrogen mustard and measuring cytoplasmic exchange in a mating of the clone derived from the treated animal with normal animals it was found that in some cases the transmission of the physical basis of reduced viability after fertilization is independent of cytoplasmic exchange between conjugant animals while in other cases cytoplasmic exchange is necessary for transmission of the basis of effects to the untreated mates. Additional evidence for cytoplasmic inheritance is summarized: the basis of reduced viability may be maintained through successive self-fertilizations and cannot be accounted for on a nuclear basis. In many experiments in which matings have been performed, the inequality of pair members after conjugation indicate that the cytoplasm is involved in inheritance of reduced viability. The level of reduced viability at fertilization is not reduced in a manner required by the hypothesis that nuclear inheritance only is involved.

GILES, NORMAN H. Jr., Oak Ridge National Laboratory, Oak Ridge, Tenn.: The occurrence of suppressors in a methionineless mutant of Neurospora. — A study has been made of the nature of adaptations (methionine independent cultures) arising spontaneously or following ultraviolet irradiation of microconidia of a methionineless mutant (original Stanford isolation number 4894). Appropriate crosses of four independently occurring adaptations have shown that these all result from suppressor mutations rather than from reverse mutations. Genetic and physiological evidence further indicates that three of these suppressors

(all of which arose spontaneously) are identical and are closely linked with albino-1 (15300) on the sex chromosome. The fourth suppressor (which appeared in microconidia treated with ultraviolet) is genetically and physiologically distinct and not sex-linked. In both types of suppressor strains, growth (as measured by mycelial dry weight) is markedly retarded as compared with wild type in a medium lacking methionine. Although one suppressor strain has a much slower growth rate than the other, both eventually attain weights equal to that of wild type even in the absence of methionine. In the presence of added methionine, the growth of both suppressor mutants is similar to that of wild type.

GINSBURG, B. E., D. S. MILLER, and M. J. ZAMIS, University of Chicago, Chicago Ill.: On the mode of inheritance of susceptibility to sound-induced seizures in the house mouse (Mus musculus). — Susceptibility to sound-induced seizures in dba line 1 mice has been attributed to a single dominant gene in test crosses to C-57 black mice of subline 6. In crosses of dba 1 to C-57 black, subline 10, the following data were obtained: (1) Seizure incidence in the $F_1$ is exactly intermediate between that of the two parent strains. (2) Seizure incidence in the $F_2$ is 3/4 that of the $F_1$. These data suggest that two or more non-dominant factors with cumulative effects are involved and that the presence of one particular "convulsing" gene may be required for seizure susceptibility. In crosses of dba line 2 to C-57 black, subline 10, the $F_1$ shows partial dominance and the seizure incidence in $F_2$ is 82% of the $F_1$. Of two sublines of dba 2 mice tested, one had a seizure incidence identical with that found for dba 1, while the other seized significantly in excess of dba 1. $F_1$ and $F_2$ animals from crosses of the latter to C-57 10 mice showed ca. 40% more seizures than the corresponding $F_1$ and $F_2$ animals from crosses of dba 1 to C-57 10. Reciprocal crosses gave identical results. The total incidence of all seizures of whatever degree of severity was taken as the index of susceptibility because all individual measures were found to be more easily affected by experimental conditions. The $F_2$ was no more variable than the parental strains or the $F_1$ when judged by the distribution of the various kinds and degrees of convulsive activities.

GLASS, BENTLEY, The Johns Hopkins University, Baltimore, Md.: The effects of supplementary treatment with infra-red radiation on X-ray induced lethals and chromosome aberrations in females of Drosophila melanogaster. — Infra-red pretreatments and posttreatments of 4 hours have been applied to ♂♂ and ♀♀ of D. melanogaster given a dose of X-rays amounting to ca. 2000 r units, the dose varying slightly for different series, but being always identical for ♂♂ and ♀♀ of the same series. Translocations induced in ♂♂ were 6 in 85 tested gametes (7.1%) when pretreated with infra-red, and 7 in 113 (6.2%) when posttreated. The differences are not significant, a fact at variance with the results of Kaufmann, who found no effect of posttreatment with infra-red. The rate in controls is about

two-thirds as great. In ♀♀, O translocations were found in 218 gametes tested when pretreated with infra-red, 1 in 169 (0.6%) when posttreated. The translocation is the first one ever found by the author in treated D. melanogaster ♀♀, in series amounting to a total of 1832 tested gametes. The salivary gland chromosomes show that this translocation involved a high number of breaks. – Sex-linked lethal mutations have been scored in both ♂♂ and ♀♀, with and without infra-red posttreatment: 15/569 (2.64%) in ♂♂ with X-rays only; 10/569 (1.76%) in ♀♀ with X-rays only; 14/281 (5.0%) in ♂♂ with X-rays plus infra-red; 2/222 (0.9%) in ♀♀ with X-rays plus infra-red. The difference between the two groups of ♂♂ and the similar difference between ♀♀ are not significant; but the difference between ♂♂ (combined) and ♀♀ (combined) is significant (2% level). The frequency of induced lethals in ♀♀ is about half that in ♂♂.

GORDON, MALCOLM, and HERSCHEL K. MITCHELL, California Institute of Technology, Pasadena, Calif.: Tryptophane desmolase in Neurospora. — As Mitchell and Lein have reported, cell free extracts of certain tryptophan requiring Neurospora mutants have no demonstrable tryptophan desmolase activity, even though extracts from the wild type, prepared identically, have such activity. It has been found that when these mutant extracts are dialyzed, fractionated with ammonium sulfate, or when the mycelium is thoroughly washed with distilled water considerable tryptophan desmolase activity can be demonstrated. However, no mutant enzyme preparation has been made, thus far, which has had more than half the activity of the wild type on a dry weight basis. The tryptophan desmolase activity of wild type extracts is not inhibited by the dialyzable or nondialyzable components of mutant extracts, or by heat inactivated mutant extracts. But activated mutant preparations are inhibited by heat inactivated wild type extracts and by the dialyzable components of such extracts. A number of compounds tested at concentrations which do not significantly inhibit the wild type preparations, are inhibitory for the activated mutant extracts. Among these compounds are ammonium sulfate and a number of naturally occurring amino acids.

GORDON, MYRON, American Museum of Natural History, New York, N. Y.: Genetics, speciation and the origin of melanotic tumors in fishes. — The macromelanophores are large black pigment cells which form a series of five distinctive color patterns in six natural populations of the platyfish Platypoecilus maculatus of the Atlantic Coast rivers of Mexico, Guatemala and British Honduras. The macromelanophore patterns are sex-linked; those from the Rios Jamapa and Coatzacoalcos have the XY male, XX female type of genetic sex determination, while those from Belize River have the XY female, XX male type. Each of the six natural populations studied statistically has a distinct frequency of the five macromelanophore patterns by which each population may be identified. These river populations have been isolated for about 300,000 years, yet though

genetically different, on morphological features of taxonomic importance they have not reached the subspecies level. When a platyfish, Platypoecilus maculatus, with the macromelanophore pattern spotted, Sp, is mated with the related genus, the swordtail, Xiphophorus hellerii, the intergeneric hybrids develop melanomas. When the spotted P. maculatus is mated with a species whose natural habitat is a thousand miles away, P. couchianus, the interspecific hybrids develop melanomas, too. When a spotted P. maculatus is mated to a member of a nearby species, P. variatus, the degree of atypical pigment cell growth is less severe in the hybrids. When a spotted P. maculatus from the Rio Coatzacoalcos is mated to the same species from the Rio Jamapa, the intraspecific hybrids show a definite melanosis. (Melanosis preceeds melanoma). The significant point is that the parents in each type of mating (intergeneric, interspecific, intraspecific) represent different genetic populations. Melanoses and melanomas in the hybrids arise in response to a genic unbalance between the genes for macromelanophores and their growth regulators, specific macromelanophore modifying genes.

GOWEN, JOHN W., Iowa State College, Ames, Iowa: Radiation effects on mice as related to survival. — The effects of acute irradiations on the subsequent lengths of life of the exposed individuals have evolutionary, as well as current, interest. Genetic variations exist in different hosts; genetic diversity is found in the organisms initiating a disease; and the environmental setting in which the disease is to occur is subject to variation. The type and character of the radiation to which either the hosts or the pathogens are exposed may be considered environmental variations. In examining the interactions of these factors, the discussion will be limited largely to the mouse and its typhoid disease caused by Salmonella typhimurium. Six inbred mouse strains of characteristically different typhoid susceptibility (8 to 90%) and leucocyte counts were exposed to X-rays in doses from 0 to 700 roentgens /cm$^2$ of body surface. All six strains reacted alike. Radiation throughout decreased resistance. The decrease in resistance was an exponential function of the dosage of X-rays to which the mouse was exposed. The data, while quite irregular due to limited numbers, show the same rate of decrease in resistance for each of the strains. Resistance at 600 r exposure was decreased to about 43% of that expected with no radiation. These results are explained by the exponential destruction of the phagocytic cells by the acute irradiation. Mice were blood counted, irradiated, and the cell counts taken again at one and two weeks after treatment. The results show a corresponding decrease in leucocyte numbers for each of the strains. The decrease, like that for survival to typhoid, is exponential.

GREEN, M. M. and K. C. GREEN, University of Missouri, Columbia, Mo.: The genetic subdivision of the lozenge locus in Drosophila melanogaster. — On the basis of their phenotypic effects the sex-linked, recessive

lozenge mutants of D. melanogaster have been considered to be multiple alleles. All affect eye color, eye structure, tarsus structure and ♀ genitalia in a similar manner. Furthermore, $\frac{oo}{++}$ heterozygous for any two lozenge mutants exhibit a lozenge phenotype. However, further genetic studies indicate that the lozenge mutants do not constitute a classical multiple allelic series. Thus from $\frac{oo}{++}$ heterozygous for the mutants $lz^{BS}$ and $lz^{46}$, in addition to the expected progeny, wild type individuals and individuals carrying both $lz^{BS}$ and $lz^{46}$ on the same X chromosome were recovered. The distribution of marker genes among the exceptional progeny indicates that equal crossing over has occurred between $lz^{BS}$ and $lz^{46}$ at a frequency of about 0.1%, with $lz^{46}$ lying to the right of $lz^{BS}$. Parallel analyses using $\frac{oo}{++}$ heterozygous for $lz^{46}$ and $lz^{8}$ yielded similar results. As a result of crossing over between $lz^{46}$ and $lz^{8}$, wild type flies and flies carrying both $lz^{46}$ and $lz^{8}$ on the same X chromosome were obtained. Crossing over here is also equal, occurs at a frequency of about 0.05% and places $lz^{8}$ to the right of $lz^{46}$. These results demonstrate that the lozenge mutants may occur in one of three closely linked loci. Using $lz^{BS}$, $lz^{46}$ and $lz^{8}$ as testers, thirteen additional mutants of spontaneous, X-ray and mustard gas origin were analyzed to determine to which locus they may be assigned. The results indicate that a total of 7 mutants may be localized at the $lz^{BS}$ locus, 6 at the $lz^{46}$ locus and 3 at the $lz^{8}$ locus; no additional loci are indicated by the data thus far obtained.

GRIFFIN, A. B., and MARY WARTERS, University of Missouri, Columbia, Mo., and Centenary College, Shreveport, La.: A new chromomeric map of the X chromosome in Drosophila melanogaster. — A new map of the salivary X-chromosome of D. melanogaster has been constructed on the basis of chromomeric structure. While Bridges' system of subdivision and numbering has been retained, the number of "lines" has been abandoned and the number of structural elements, the chromomeres, emphasized as the basis for cytogenetic investigations. Each section of the chromosome is presented in numerous drawings taken from various wild stocks and from different aceto-carmine fixations; these duplicates are aligned below the final composite map so as to provide a convenient means for recognizing the inevitable differences in the appearance of average drawings and the standard map.

GRIFFEN, A. B., MARY WARTERS, and JAMES D. MOHLER, University of Missouri, Columbia, Mo., and Centenary College, Shreveport, La.: The position effects Bar, white-mottled, and cubitus interruptus in Drosophila melanogaster. — Comparisons of X-ray induced reversals of position effects at the Bar, white, and ci loci indicate that each locus may revert to wild type action either by mutation or by virtue of new chromomere associations resulting from new chromosome rearrangements. In the case of $B^{s}$, mutation accounts for approximately one-third of the phenotypic changes from $B^{s}$ to wild type.

GRIFFING, BRUCE, Iowa State College, Ames, Iowa: Use of variance components with constant parent regression technique in estimating quantitative gene action. — The problem under consideration was to estimate the simplest gene model which would accurately fit the observed results in tomato fruit size inheritance. Six inbred lines covering the range of fruit weights from 0.5 to 142.6 grams were combined in all possible combinations to give fifteen $F_1$'s. The experimental design was that of six randomized blocks with seven plants per plot which totaled forty-two observations for each inbred of $F_1$ mean. The experiment was conducted in the field during the summer of 1947. The fitting of a gene model was accomplished by contrasting statistical analyses of the data in both arithmetic and logarithmic scales of measurement. The statistical approaches involved: (1) constant parent regression trends, (2) partitioning of $F_1$ genotypic variance into that attributable to regression and deviations from regression and, (3) components of $F_1$ genotypic variance which involve basic gene and dominance components and covariance of basic gene and dominance effects. Logarithmic scale was chosen as best fitting scale. The estimate of the dominance value ($h \cong .20$) attached to the logarithmic scale was determined by the constant parent regression trend, and variance ratios together with potence ratios for the extreme parental groups.

HAAS, F., J. B. CLARK, O. WYSS, and W. S. STONE, The University of Texas, Austin, Texas: Mutations and mutagenic agents in bacteria. — Previous work has shown that in Micrococcus aureus the incidence of mutation to resistance to penicillin and streptomycin can be increased by growing the microorganism in a substrate which has been irradiated with ultra-violet light or treated with hydrogen peroxide. The same techniques of treatment have been applied with success to a different organism and with regard to a different mutation: that of increasing the mutation rate to bacteriophage resistance in various strains of Escherichia coli. Some of the properties of phage resistant biochemical mutants obtained by this method are compared with those phage resistant biochemical mutants occurring spontaneously. Certain chemicals will also increase the incidence of mutation in Micrococcus aureus and Escherichia coli when these organisms are grown in their presence. The temperature at which the substrate is irradiated produces an effect on death rate and subsequent incidence of mutation. Some effects of visible light and infra-red radiation on bacterial killing and the incidence of mutation in bacteria subjected to direct irradiation with ultra-violet are compared with those effects obtained in bacteria grown in treated substrates.

HERSKOWITZ, IRWIN H., School of Medicine, Louisiana State University, New Orleans, La.: Tests for chemical mutagens in Drosophila using the larval feeding technique. — Various factors are being studied for their effect on the mutagenic potency of chemical substances. Under investigation are the technique whereby the chemical is administered, the type of cell

studied and its stage in differentiation, and the metabolic rate. As part of this plan 2 4-dinitrophenol and methyl bis (β -chloroethyl) amine hydrochloride (N mustard) alone and in combination were placed on the food (50 cc. per bottle) in which Drosophila melanogaster eggs and larvae up to 48 hours old were developing. These substances were chosen because of the effect of the former on cellular metabolism and of the latter on mutation rate. The occurrence of sex-linked recessive lethals in individuals completing development in the presence of sub-lethal concentrations of the chemicals were determined by means of the Muller-5 technique. The results obtained follow: 50-100 mg. 2,4-dinitrophenol, 6 lethals in 2241 X chromosomes tested; 1-2 1/2 mg. N mustard, 8 lethals in 1646 tests; 20 mg. 2,4-dinitrophenol plus 1 1/2 mg. N mustard, 4 lethals in 2942 tests. These findings are not regarded as showing an increase in mutation rate over the normal. (Work supported by a grant from the National Cancer Institute, U. S. Public Health Service.)

HIMES, MARION, and CECILIE LEUCHTENBERGER, Columbia University, New York, N. Y.: The effect of some folic acid analogues on mitosis. — It has been shown that folic acid is probably of importance in the synthesis of desoxyribose nucleic acid and that a folic acid analogue, 4-amino-pteroylglutamic acid, has a potent anti-folic acid activity (Oleson, Hutchings and Subbarow, reference). Therefore it seemed of interest to investigate cytologically the effect of folic acid analogues on mitosis. The following analogues, which were synthesized and supplied by the Lederle Laboratories, were tested as to their effect on the mitosis of onion root tips: 4-amino-pteroylglutamic acid (aminopterin), 4-amino-$N^{10}$methyl-pteroylglutamic acid (a-methopterin), 4-amino-pteroylaspartic acid (amino-an-fol), and pteroyltriglutamic acid (teropterin). The onion root tips were exposed to concentrations varying from 0.2 - 2 mg./cc. for 1 -24 hours. Controls and treated root tips were fixed in Carnoy, embedded in paraffin, and sections stained with Feulgen and methyl green. The results showed that if onion root tips were treated with aminopterin, no late stages of mitosis occurred; all the meristematic nuclei appeared to be in a stage which resembled an early prophase or an abnormal interphase. In contrast to these results, onion root tips exposed to the other three analogues (a-methopterin, teropterin and amino-an-fol) showed late stages of mitosis similar to the controls. The effect of aminopterin on mitosis is inhibited if any one of the three other analogues is added to the aminopterin solution.

HSU, T. C., University of Texas, Austin, Texas: Cytological studies in the virilis group of Drosophila I. Inversion variations in the americana-texana-novamexicana complex. — In the virilis group of Drosophila, Drosophila novamexicana has five pairs of rod-chromosomes and a pair of dots. D. americana texana has the chromosomes 2 and 3 fused; and D. americana americana has an X-4 fusion in addition to the 2-3 fusion.

Using the salivary map of virilis as standard, novamexicana has the following inversions: Xabc, 2abc, 3a, 4a, 5b; texana has Xab, 2a, 5a. The distribution of novamexicana is in Arizona, New Mexico and Colorado; and that of texana is in the southeastern United States. D. americana has two branches: one distributed along the Missour River Valley up to Montana, and the other along the Ohio River Valley up to New York. The inversions of the western branch are similar to novamexicana, being Xabc, 2ab, 4ab, 5b (one strain is also heterozygous for 3a). Some strains have additional inversions Xd and 4c. The eastern branch is similar to texana with the exception of the X which is Xabc. It has been suggested that americana has arisen from hybridization between novamexicana and texana in Central Texas and spread from there along the Mississippi River. Collection data show that its eastern branch has a long, belt-like overlapping zone with texana where natural hybrids occur. The western branch may resemble novamexicana in the inversion contents by repeated crossing but no overlap zone is known.

HUSKINS, C. L., and L. CHOUINARD, University of Wisconsin, Madison, Wis.: Somatic reduction: Diploid and triploid roots and a diploid shoot from a tetraploid Rheo. — From two tetraploid Rhoeo discolor plants (obtained from R. E. Clausen and grown in Hoaglund's solution) 168 tetraploid, 13 triploid, and 7 diploid main roots and one diploid shoot have been obtained. Many roots predominantly tetraploid had patches of triploid and diploid cells. Somatic reduction ("reductional grouping") of chromsomes was studied in tetraploid and triploid roots untreated and treated with sodium nucleate which greatly increases its frequency. Groupings of 12 , 12 and 6 : 18 occur in the tetraploid and of 9 : 9 and 6 :12 in the triploid with greater than random frequency. The excess of equal distributions might be explained by mere mechanical causes but some type of repulsion between homologues and/or selective survival is suggested by the excess of 6 : 18 and 6 : 12 since the former could have and the latter alone conceivably can have complete sets of homologues, as the triploid and diploid roots also probably have. Differences between two groups in mitotic stage or plane of chromatid separation are taken to indicate that the reduction took place in an antecedent cell, which would account for their greater frequency in controls.

HUTT, F. B., Cornell University, Ithaca, N. Y.: Semi-lethal action of the gene for extension of black pigment in the fowl. — The popular sex-linked cross, Barred Rock $\frac{\varphi\varphi}{ZW}$ X Rhode Island Red $\frac{ZZ}{\delta\delta}$, usually yields a number of unsalable chicks described as "bare backs." These have sparse down or none at all over varying amounts of the dorsal surface. Such chicks are always females. Studies have shown that this abnormality is attributable, not to any sex-linked or sex-limited mutation, but to a pleiotropic effect of the gene E for extension of black pigment. Solid-black chicks have shorter down feathers than those in which the black is reduced by genes for

barring or for restriction. From this cross, chicks of both sexes have black down but the (non-barred) females have shorter down than the (barred) males. In extreme variants the down is absent, or so short that the chicks appear bare. The semi-lethal action of E is proven by a significant deficiency of females among chicks hatched from this cross and by an excess of females with "clubbed," short down among those that die in later stages of incubation. Bare-backed male chicks can be obtained from matings that yield solid-black, non-barred chicks of that sex. From diallel crosses it was found that the down is longer and "barebacks" fewer in black chicks carrying the gene k for rapid feathering than in those with its allele K for slow feathering. The bare-backed chicks can therefore be almost entirely eliminated by using for this cross males of the genotype kk.

IVES, PHILIP T., Amherst College, Amherst, Mass.: Non-random production of visible mutations by the Florida high mutation rate gene in Drosophila. — Of 42 sex-linked visible mutations occuring incidentally in several years tests of the sex-linked lethal mutation rate which obtains in lines of flies carrying the Florida second chromosome high mutation rate gene, high, only one was a yellow allele while four were alleles of the infrequent mutant, folded. During the same time, many sex-linked visible mutations occurred incidentally in a large number of stocks of different second chromosome lethals balanced with Curly. During this past year these latter mutations number 37, of which 19 were alleles of yellow and none were folded. Both series of visibles included one or two alleles each of other well-known mutations. It is suggested that mutation rate genes may be important factors in controlling the kind of visible mutants which occur, and that they represent a possible mechanism for paleontologically rapid structural changes in organic evolution.

JAMES, ALLEN P., Iowa State College, Ames, Iowa: Pause in Lactobacillus arabinosus adaptation to tyrosine deficiency. — Inoculation of a tyrosine-deficient synthetic medium with a normal strain of Lactobacillus arabinosus 17-5 may result in the natural occurrence of tyrosine-independent strains. The deficient medium permits a limited growth of normal cells. The level of this growth is affected by the concentration of phenylalanine present and by the length of autoclaving of the medium. Periodic cell counts of samples of 'adapting' cultures show that mutants are most likely to arise during the initial growth of the normal cells. However, the growth curve of these mutants is frequently characterized by an intermediate stationary phase of indeterminate length. Resumption of cell division results in high turbidity of the cultures. As a consequence of this pause in growth, 'adaptation' is delayed, and concentration of mutant cells at a specific time cannot be used to estimate time of occurrence of mutation within cultures. Attention has been focused on the resumption of growth of the mutant cells with its indication that adaptation to tyrosine-deficiency is the result of successive step-mutations.

KALISS, NATHAN and GEORGE E. JAY, Jackson Memorial Laboratory, Bar Harbor, Maine: The use of inbred strains of mice to study the presence of iso-antibodies. — Gorer has shown that when tumor 15091a, a transplantable adenocarcinoma indigenous to inbred strain A mice, is inoculated subcutaneously into C 57 black mice, an antibody is produced by these mice which will agglutinate strain A red cells. Gorer concluded that tumor and red cells have an antigen in common. However, the antiserum might have been evoked by A red cells inoculated along with the tumor. Accordingly, we adopted two procedures to separate red cells from tumor before inoculating the tumor into C 57 black mice. (1) Tumor 15091a was inoculated subcutaneously into Street mice, genetically unrelated to A mice. The tumor grows progressively in 50% of Street mice. Such tumors, now containing Street red cells, were inoculated into C 57 black mice. Antiserum from the black mice agglutinated A red cells, but did not agglutinate Street red cells. (2) Tumor 15091a was inoculated subcutaneously into C 57 black mice which had previously received injections of lyophilized tumor 15091a. The tumor grows progressively in such mice, whereas it regresses in 100% of untreated blacks. These tumors, containing C 57 black red cells, were inoculated into untreated black mice. No agglutinin for A red cells could be demonstrated in serum of the latter mice. (Work supported in part by a grant-in-aid from the American Cancer Society, upon recommendation of the Committee on Growth of the National Research Council, and in part by a grant to the Roscoe B. Jackson Memorial Laboratory from the National Cancer Institute.)

KAUFMANN, BERWIND P., HELEN GAY, and MARGARET McDONALD, Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Analysis of cell structure by digestion with enzymes. — Treatment of sections and smears of fixed cells with purified enzymes (ribonuclease, desoxyribonuclease, pepsin, trypsin, and chymotrypsin), used independently and in succession, has permitted identification of nucleic acids and proteins and determination of their location and patterns of association. Experiments in which basic and acidic dyes were used to determine the results of enzymatic degradation indicate that part of the ribonucleic acid exists in association with a tryptophane-containing protein, and part of the desoxyribonucleic acid in association with histone. Degradation of either of these complexes does not result in cellular destruction or dissolution of the chromosomes. Neither histone, nor tryptophane-containing protein, nor nucleic acid in itself seems to be the essential material for maintenance of structural integrity. Additional support of this interpretation is provided by analysis of the course of tryptic digestion of root-tip cells and salivary-gland chromosomes. When used in buffered solution, trypsin causes cellular dissolution. When it is used in aqueous solution at pH 6, no visible alteration of structure occurs, although there is enzymatic action as judged by alteration of staining patterns. Subsequent treatment with electrolytes and then water causes swelling of the cell and deformation or loss

of nucleus and chromosomes. Experiments in which aqueous solutions of trypsin were used before or after other enzymes have sustained the interpretation that the chromosome is an integrated fabric in which no single protein or nucleic acid may be regarded as the primary structural component.

KIMBALL, R. F., and NENITA T. GAITHER, Oak Ridge National Laboratory, Oak Ridge, Tenn.: Photorecovery of the effects of ultraviolet radiation on Paramecium aurelia. — Four different effects of ultraviolet radiation and one of X-rays upon Paramecium aurelia have been examined for the action upon them of intense illumination with a lamp (GE H-5) having its major output in the long ultraviolet and short visible. The animals were exposed to ultraviolet of wave length 2650 Å or 2804 Å or to X-rays and immediately afterwards exposed to the H-5 lamp. Except during this exposure, dark conditions were maintained as far as possible. The effects studied were as follows: (1) mutation as measured by death and reduced division rate after autogamy; (2) temporary reduction in division rate in the first few days after irradiation and in the absence of autogamy; (3) death, presumably nongenic, in the first few days after irradiation and in the absence of autogamy; (4) a microscopically visible change in the structure of the macronucleus requiring a few hours to reach complete expression. In all four cases, light from the H-5 lamp greatly reduced the effects produced by the shorter ultraviolet. The induction of mutations by X-rays was not affected. The fact that light from the H-5 lamp affects several kinds of changes produced by short ultraviolet suggests that it may act upon some process basic to all the changes. The failure to detect an effect upon X-ray induced mutations suggests that they may be different in some way from those induced by ultraviolet.

KING, R. C., Yale University, New Haven, Conn.: Genetic changes accompanying the uptake of $P^{32}$ by Drosophila melanogaster. — Adults or larvae of Drosophila melanogaster are found to incorporate large amounts of $P^{32}$ into their tissue after feeding on yeasted cornmeal-molasses-agar media to which various amounts of $H_3P^{32}O_4$ had been added previously. The feeding of adult males seems to be the most efficient means for producing genetic changes, since females given the same treatment show lower percentages of sex-linked lethals than males. Drosophila in developmental stages are very sensitive to radiation and fail to survive such treatments as would result in very low rates of mutant induction for adults. $P^{32}$ is distributed among the head, thorax, gut, gonad and abdominal shell in the ratio of 20:50:10:10:10 for males fed as adults on the $P^{32}$-containing medium regardless of the total activity of the fly. Radioautographs made of the spermathecae and associated organs of untreated females fertilized by treated males gave direct evidence that the sperm were radioactive. Some treatments have produced sex-linked lethal mutation rates as high as 26% as measured in adult males

of an inbred Canton-S stock by the M5 technique. Among a combined total of some 1,951 treated X's so far tested from all experiments 90 sex-linked recessive lethals and 11 viable mutants have been recovered. By changing the initial activity-feeding time relations or by increasing the number of $P^{31}$ atoms in the medium while keeping the number of $P^{32}$ atoms constant it is possible to expose different groups of flies to an equal flux of high energy beta particles from the medium but to an entirely different internal radiation dose from the radioactive decay of $P^{32}$ atoms incorporated into tissue. By determining the dominant and sex-linked recessive lethal mutation rates induced by treatments of this sort, it may be possible to determine what part of the genetic changes accompanying $P^{32}$ uptake is due to the recoil occurring during the transmutation of $P^{32}$ incorporated into chromosomes to $S^{32}$. Perhaps some modification of the curie-equivalent roentgen equations may be necessary.

KRIVSHENKO, JAKOV, University of Missouri, Columbia, Mo.: On the genetical activity of the Y chromosome in Drosophila buscki. — In studying primary non-disjunction in D. buscki, exceptional males never occurred. As special crosses demonstrated that O-gametes are viable and capable of fertilization, we conclude that male imagos are not formed unless a Y be present. Six Y-associated mutations obtained by X-raying have been investigated, five of them involving translocations between Y and autosomes. In the progeny of two of these mutants, Minute-Y and Delta-Y, two types of males appear, one mutant, the other approaching wild type. The last class lacks part of the Y, but is hyperploid for small parts of autosomes. In Minute-Y, the Y being broken close to its proximal end, hypoploid males are few (11%), of low viability and sterile. In Delta-Y the break is beyond the enchromatic part of the Y; here hypoploid males are fairly numerous, viable and fertile. Evidently a small part of the Y suffices to give sterile male imagos of low viability, but the whole enchromatic part is needed for normal males. The sixth mutation is free of aberrations, and its effect is the suppression of the autosomal recessive cherry in males. In this line, some normal-eyed females appeared and they proved to have a Y chromosome. Finally YY males, one Y carrying the mutant and the other +, also showed suppression of cherry. Thus the mutant is a dominant suppressor. All these data show that the Y chromosome in D. buscki, unlike all other species of Drosophila hitherto studied, is undoubtedly genetically active and contains important genes necessary for the development of male zygotes.

LEDERBERG, JOSHUA, University of Wisconsin, Madison, Wis.: Segregation in Escherichia coli. — Hitherto, gene segregation and recombination in Escherichia coli K-12 has necessarily been studied exclusively in the limited, prototrophic recombination class which is selected by minimal medium. These studies supported the concept of a single linear linkage group as the basis of gene recombination in the haplobiontic life

cycle of this bacterium. Recently, K-12 substrains were found which produced heterozygotes that could be maintained as diploids, but which could be allowed to segregate freely on a complete, unselective medium. Therefore, all segregants can be recovered without restrictions imposed by nutritional selection hitherto necessary. Segregation was found to be highly aberrant, with factorial ratios deviating widely from unity. The deviations can be interpreted by a process whereby a segment of chromosome contributed by one parent is regularly eliminated from the diploid zygote, resulting in an aneuploid cell, in which some factors are haplogenic. The deficient segment behaves as a recessive lethal, so that half the segregants are inviable. This segment contains factors for fermentation of maltose and galactose, and for resistance to streptomycin, which, therefore, cannot be placed on the linear linkage map. It is still undetermined whether the elimination mechanism is a unique result of structural heterozygosity contributed only by certain strains used as parents, or whether it reflects an innate instability of the bacterial chromosome.

LEFEVRE, GEORGE, Jr., University of Utah, Salt Lake City, Utah: X-ray induced genetic effects in germinal and somatic tissue of Drosophila melanogaster. — The rate of X-ray induced direct mutation was compared following irradiation of germinal and somatic tissue. Various data regarding the frequency of mutation to white after exposure of adult $w^+$ males indicate that the average germinal mutation constant for the mutation of $w^+$ to $w$ is about $12.5 \times 10^{-8}$. Possible errors inherent in the estimation of a somatic mutation constant can be evaluated, and by employing proper techniques reliable data on the somatic mutation rate of $w^+$ to $w$ can be obtained. From the incidence and average size of white eye-color mosaics produced by irradiation of + male larvae, a somatic mutation constant of $14.2 \times 10^{-8}$ was calculated. Thus, X-ray induced mutability of the $w^+$ locus is not significantly affected by the kind of cell in which it is located. Attempts were made to induce reverse mutations in both germinal and somatic tissue. No evidence of reverse germinal mutation was found following irradiation with 5000 r of 166,000 recessive X-chromosome loci, including y, w, $sn^3$, and f. In somatic studies no reverse mutations of white were found in tests equivalent to the exposure to 5000 r of 600,000 w alleles of three diverse origins: (a) spontaneous, (b)X-ray induced, and (c) mustard gas induced. Serious question is thrown on the reliability of early reports of X-ray induced reverse mutation in Drosophila. A comparison of the published data regarding the influence of various intrinsic and extrinsic factors on X-ray induced and spontaneous mutation suggests that the two mutation processes are qualitatively different.

LEIN, JOSEPH and PARTICIA S. LEIN, Syracuse University, Syracuse, N.Y.: Genetic control of fatty acid synthesis in Neurospora. — Using the selection technique developed by Lein, Mitchell and Houlahan,

Neurospora crassa mutants requiring unsaturated fatty acids for growth were specifically sought for and obtained. One of these mutants grew in oleic acid, linoleic acid and linolenic acid but not in saturated fatty acids. Genetic tests revealed Mendelian segregation of the fatty acid requirement, thus indicating that these fatty acids are biosynthetically related. Although these fatty acids enable growth, they also inhibit growth at higher concentrations. This inhibition can be at least partially relieved by the synthetic wetting agent, Tween 60. A second mutant was found to grow in saturated as well as unsaturated fatty acids, indicating synthesis of the latter from the former. Through study of this and other fatty acid mutants, it is hoped that the pathway of synthesis of saturated and unsaturated fatty acids will be elucidated.

LENG, EARL R., University of Illinois, Urbana, Ill.: Inheritance of number of kernels per row in corn. — Heritability of number of kernels per row in corn has been found to be approximately twice as high as that of ear length. Regression analysis has given evidence that dominance relationships of genes affecting number of kernels per row varies between different inbred lines. The low kernel-number inbred lines 5120B and Hy2 perform very differently in crosses. 5120B appears to carry mostly recessive factors for this character, while Hy2 shows virtually complete dominance. Morphological studies have shown that Hy2 is homozygous for a staminate ear tip, which is inherited as a complete dominant. It appears that there may be a relation between this character and number of kernels per row.

LENG, EARL R., C. M. WOODWORTH, and R. J. METZGER, University of Illinois, Urbana, Ill.: Estimates of heritability and degree of dominance in certain quantitative characters of corn and soybeans. — Estimates of heritability and dominance relationships have been made by regression analysis of parent-$F_1$ data in both corn and soybeans. Seven sets of data have been analyzed in corn and one in soybeans. Characters considered in corn have included ear length, number of kernels per row, row number, kernel size, and grain yield. The chief characters studied in soybeans have been branch number, node number, pod number, total seed number, seed size, and number of seeds per pod. Estimates of heritability were rather low for most of the characters considered in both crops, except for number of kernels per row in corn and seed size and number of seeds per pod in soybeans. Possible over-dominance effects indicated in analysis of total yield were not found when components of yield were considered individually. The data on seed size and number of seeds per pod in soybeans closely fitted theoretical results expected on the basis of additive gene action.

LIEB, MARGARET, Columbia University, New York, N.Y.: Forward and reverse mutation rates of biochemical mutants of bacteria. — Mutation

rates are being studied in populations of various sizes of E. coli, strain 15 (Roepke). The rate of spontaneous back mutation from histidine-requirement (h-) to histidine-independence (h+) is of the order of $5 \times 10^{-8}$ per division cycle. Preliminary results indicate that there is no phenotypic lag. The mutation rate of h+ to h- has been determined using a modification of the penicillin selection method originated by Davis, and Lederberg and Zinder. A known number of h- cells, genetically marked, was added to a population of h+ cells prior to penicillin treatment. The number of marked cells surviving together with the number of new h- cells found after treatment was used to calculate the number of spontaneous h- present prior to the addition of penicillin. The spontaneous mutation rate of h+ to h- is approximately $7 \times 10^{-7}$ or about ten times the rate of back mutation. The absence of recombination in this strain makes it difficult to determine whether these mutations involve one or many genes.

LURIA, S. E., Indiana University, Bloomington, Ind.: Type hybrid bacteriophages. — The coliphages T2, T4, T6 are morphologically identical and serologically cross-reacting although distinguishable. Genetic relationship is proved by the occurrence of genetic recombination in the progeny of type crosses (Delbrück and Bailey). The wild-types differ among other things in serological specificity, host range, cofactor requirements and sensitivity to ultraviolet. The study of these characteristics in hybrids may help in interpreting the relation between wild-types. Taxonomically useful characters are: (1) serological specificity (not yet found to change by mutation); (2) host range (each type of a pair being characterized by activity on one and inactivity on another one of a pair of bacterial mutants; inversion of this pattern should require at least two mutations). In crosses T2 x T4 these two criteria have given consistent results and permitted characterization of each hybrid as either T2 or T4. UV sensitivity levels (wild-type T2 being twice as sensitive as T4) are distributed without intermediate levels among both hybrids T2 and T4 and have, therefore, little taxonomic value. The same is true of cofactor requirements, which can be altered by mutation (Delbrück) or recombination (Hershey). Host range of T2 is affected by mutations T2-T2h restoring activity on bacterial mutant B/2 without suppressing activity on B/4 or B/6. Crosses T2h x T4, T2h x T6 yield hybrids T2h+, indicating the presence in T4 and T6 of the wild-type allele of the h property (only detectable in T2). This system is used in analyzing the phage mutation T2-T2h and the bacterial mutation B-B/2.

MACFARLANE, EILEEN W. E., Institutum Divi Thomae, Cincinnati, Ohio: Somatic mutations produced by organic mercurials in flowering plants. — Young seedlings of Raphanus and Zea were treated with pehnyl mercuric hydroxide and basic phenyl mercuric nitrate 1 part in 10,000 tap water for 6 hrs. and 18 hrs. respectively. Subsequently some leaves

developed with: (1) larger stomata than the controls and with patches of enlarged epidermal tissue. (2) Sectorial chlorophyll mutations: yellow variegation appeared in leaves of Zea after treatment of the stem apex by the wick method with PMOH 1 : 50,000 and 1 : 100,000 for 3 days, in the 6-leaf stage; albino tissue prominent in the sheaths of the inflorescence in one of these same Zea seedlings; an albino lateral shoot developed in Ruppia from a shoot, after an hours treatment in PMOH 1 : 100,000 which killed the leaves and most of the stem. (3) A nana type lateral shoot developed from an Elodea shoot treated along with the Ruppia. (4) Pollen sterility: Raphanus plant from the treated seedlings and a Coleus flowering shoot grown in PMOH 1 : 1 1/4 million for 8 days, showed 50 percent and 30 percent dwarf pollen grains respectively, with most of these adhering in tetrads. (5) Polyploid nuclei, sticky chromosomes and chromosome fragments were observed in a few dividing cells in root tips of Allium cepa after exposure to high dilutions of these mercurials.

METRAKOS, J. D., McGill University, Montreal, Canada: The effect of fostering on the growth pattern of the house mouse. — Three strains of mice, designated here as "Large," "Small," and "Intermediate" because they breed true for large, small, and intermediate size, were employed to study the effect of fostering on the growth pattern of the mouse. Litters were reduced to two females and two males each, and were either left on their own mother or fostered on a female from one of the other two strains. The weights of all mice were recorded every two days up to 60 days, and every four days up to 140 days. The fourteen-day mean weight of mice which had received milk from "Large" mothers was significantly larger than that of mice which had received milk from either the "Small" or "Intermdeiate" strains. In general, these differences tend to remain, but the majority of them are not statistically significant at 140 days. It is, therefore, concluded that the differences in the adult weight between "Large," "Small," and "Intermediate" strains are due primarily to differences in the genotype and not to differences of the milk supply. However, the role of the milk supply of the suckling young can modify greatly the pattern of the early growth periods. At about fourteen days when the differences are greatest, non-fostered and fostered mice of the same strain may differ in weight by as much as forty percent.

MEYER, HELEN U., MARGARET EDMONDSON, LUOLIN ALTENBURG and H. J. MULLER, Indiana University, Bloomington, Ind.: Studies on mutations induced by ultraviolet in the polar cap of Drosophila. — Studies of fertility and pre-imaginal mortality of flies derived from embryos whose polar caps were irradiated with "germicidal" ultraviolet (chiefly 2537Å) showed that dechorionation permits about ten times as much penetration of this light. While changing the angle of incidence on the dechorionated polar cap from side illumination ($0^{o}$-$20^{o}$ to plane of cap) to $45^{o}$ increased these effects several fold, changing from $45^{o}$ to $90^{o}$ increased them only some 40% more

— an indication that most polar cells are effectively reached at 45°. At this angle (that of most of our experiments on mutagenesis) increase in dosage caused decrease in the number of polar cells forming functional germ cells, as shown by the increased proportion of offspring carrying any given mutation. The mutations looked for were second chromsome lethals and partial lethals, though many genes with visible and/or sterilizing effects were found also. None included translocations. Some five times as many complete lethals occurred as those between 0% and 10% viability. Largescale tests, participated in by S. L. Campbell, showed that both types have appreciable dominance, averaging several percent. There were, however, a few with much stronger dominance. Several genes causing the heterozygous female to produce inviable embryos were found. A few tests for weakly detrimental, invisible mutations indicated their occurrence to be relatively frequent. Numerous mutations acted as recessive lethals at warm but not cool temperature, or vice versa. The distribution of mutational types was sensibly the same at all doses. Of eleven specially analyzed second chromosomes carrying induced lethals, ten were found to contain additional mutations. (Work supported in part by grants from U. S. Public Health Service, on recommendation of National Advisory Cancer Council).

MICKEY, GEORGE H., Northwestern University, Evanston, Ill.: Abnormalities of the reproductive system in Drosophila melanogaster. — A low percentage of Drosophila males from different stocks show external genitalia completely doubled. The anal plates, genital arches, claspers and penis are all fully formed and duplicated. Generally the genital structures lie on opposite sides of the anus but may form a V-shaped arrangement. They may lie in a vertical plane or show varying degrees of rotation. Internal organs also are doubled; the posterior and anterior ejaculatory ducts and sperm pumps are always completely duplicated, whereas the accessory glands, vasa efferentia and testes exhibit different degrees of fusion. Most often the testes are reduced or rudimentary, but frequently the pair on each side are fused. Occasionally three testes are fused leaving one separate; very rarely all four fuse. Although mature sperm are generally present, no specimen has proved fertile, probably because of mechanical difficulties of copulation. In females supernumerary spermathecae sometimes occur. Several cases of three and one case of four have been observed. The extra spermatheca may grow on the same stalk with another, but in one case it was attached to the anterior end of one of the paired oviducts at the base of the ovary. These instances of abnormal reproductive systems offer clues toward the understanding of normal development of reproductive structures in Drosophila.

MITTLER, SIDNEY and ROBERT H. HERMAN, Illinois Institute of Technology, Chicago, Ill.: Disruption of mitosis in onion root cells by

thyroid powder. — Numerous substances have been reported to disrupt onion root mitosis. However, the majority of them were not found in living organisms. With this in mind several organic substances that normally occur in mammals were used, namely acetylcholine, adrenaline, insulin, parathyroid powder, dried thyroid tissue, and histamine. These substances were made in concentration from 1 mg. to 10 gm./100 ml. of $H_2O$. The onion (Allium cepa) roots were treated from 4 to 72 hrs. Thyroid powder (desiccated thyroid tissue) was the only one that produced abnormal mitotic figures: polyploidy, c-mitosis, nondisjunction, and chromatin bridges. No abnormalities were found in roots treated with less that 130 mg./100 ml. of thyroid tissue. The most common abnormality found with concentrations above .7 gm. was c-mitosis, while at lower concentrations chromatin bridge formations were the most frequent. Roots treated with thyroxine (sodium salt) ranging from 1 to 40 mgm./100 ml. of buffer at pH 7.8 had normal mitotic figures. Thryoxine as the causative agent can be ruled out. The concentration of thyroid tissue used to produce abnormal mitotic figures does not contain enough ribose nucleic acid to account for the observed abnormalities. There is an unknown water soluble factor in desiccated thyroid tissue, which appears to increase upon standing in the refrigerator, that produces abnormal mitotic figures in the onion root.

MULLER, H. J., J. I. VALENCIA and R. M. VALENCIA, Indiana University, Bloomington, Ind.: The frequency of spontaneous mutations at individual loci in Drosophila. — Spontaneous visible mutations involving 9 "chosen" sex-linked loci (prune, white, ruby, carmine, singed, raspberry, vermilion, garnet, forked) were looked for among approximately 60,000 non-disjunctionally produced daughters of females having one Y-chromosome, one X-chromosome with all these genes mutant and one with inversions but all these genes normal. Nine verified "gene-mutations" and an estimated six that were not analyzed arose at these loci, scattered as though randomly. Thus the frequency of spontaneous gene-mutation in the female here was 1 in 36,000 ($\approx 3 \cdot 10^{-5}$) per locus. But since females of the parental stock gave nearly 0.7% sex-linked lethal mutations, a rate almost quadruple the usual, the frequency of gene-mutations at the 9 loci would ordinarily average between $10^{-5}$ and $7 \cdot 10^{-6}$ per locus in females. In a different stock, homozygous for cinnabar to facilitate detection of eye color changes, L. & E. Altenburg found none among 50,000 sons. Allowing for inviability here, this falls significantly below $3 \cdot 10^{-5}$ but not below $10^{-5}$ per locus. In our own material, miniature and cut mutations were detectable also. However, low viability rendered miniatures unreliable (none appeared). Six cuts, those analyzed being cytologically invisible lethals, probably gene-mutations, arose among the 60,000, i.e. $10^{-4}$. In addition, 18 to 21 mosaic mutations involving one of the "chosen" 9 loci, 4 involving miniature (more viable when mosaic), and 4 cut, were found. None proved transmissible. Hence most had arisen

during embryogeny. No gross rearrangements were found. Three mutants (all white lethals) were designated presumptive deficiencies. Two were examined cytologically, one proving invisible, the other obviously deficient. (Work supported by grant from American Cancer Society, recommended by Committee on Growth of National Research Council).

MULLER, H. J., R. M. VALENCIA, and J. I. VALENCIA, Indiana University, Bloomington, Ind.: The production of mutations at individual loci in Drosophila by irradiation of oocytes and oogonia. — To study mutagenesis in interphase nuclei females undergoing semi-starvation were X-rayed with 4600 r and then passed through a series of cultures, usually at 4-day intervals. Offspring from first cultures represented irradiated later oocytes, with chromosomes relatively condensed, and those from later cultures "resting" oogonia or, more rarely, early oocytes, with chromosomes attenuated. The genetic technique described in our parallel paper on spontaneous mutation was used, mutations at the 11 specified loci being looked for in non-disjunctionally produced daughters. 8,865 such daughters from the first cultures were observed, 94,380 from later cultures. Over 200 mutants were obtained, and about 100 bred and analyzed cytogenetically. In the attenuated chromosomes, gene-mutations were induced with a frequency apparently somewhat below but at least half as high as that previously obtained from spermatozoon irradiation. Presumptive deficiencies were however far less abundant than from treated spermatozoa. Their frequency, in relation to gene-mutations, seemed like that in the untreated material. Only 2 of 9 presumptive deficiencies examined proved cytologically visible. None of the mutations analyzed involved gross rearrangements. The more condensed chromosomes (later oocytes) gave frequencies of both gene-mutations and presumptive deficiencies sensibly similar to results from treated spermatozoa. For deficiencies, the difference from oogonia was highly significant. But again, gross rearrangements were absent. Chromosome breakage evidently occurs as frequently here as in spermatozoa, but union of fragments must occur fairly promptly, i. e. prior to the movements of the meiotic divisions. Mosaid mutants appeared with only the spontaneous frequency, there being no evidence of delayed mutation. (Work aided by grant from American Cancer Society, recommended by Committee on Growth of National Research Council).

NEWCOMBE, H. B. and M. H. NYHOLM, National Research Council of Canada, Atomic Energy Project, Chalk River, Ontario, Canada: Crosses with streptomycin resistant and dependent mutants of Escherichia coli. — Streptomycin resistant (SR) and dependent (SD) mutants of E. coli produced by ultraviolet irradiation have been crossed with the sensitive (SS) form and with each other (using Lederberg's strains Y-40, Y-53, W-677 and 58-161). The streptomycin response of at least 90 percent of the progeny resembled that of the biotin and methionine independent parent, this being

true of all crosses. Streptomycin response is also linked with the fermentation of maltose, xylose, galactose, and arabinose, but not in a manner implying linear arrangement of the genes concerned. Crosses of SR x SD have given no SS segregants, which suggests that the genes concerned are mutants either of a single locus or of two closely linked loci. In addition sensitive and resistant mutants derived from the SD form by u.v. irradiation (SD/SS and SD/SR) have been crossed with SS and SR strains. Three out of four such double mutants failed to give SD segregants, indicating either that a single locus has taken part in both mutations, or that the second mutation occurred at a closely linked modifier locus. In the one double mutant which yielded SD segregants on crossing there was evidence of a modifier gene which was linked with the locus ($Lac_1$) for lactose fermentation.

NIGON, VICTOR and ELLSWORTH C. DOUGHERTY, Faculté des Sciences P.C.B., Paris, University of California, Berkeley, Cal., and California Institute of Technology, Pasadena, Cal.: The first morphological mutant described in the Nematoda. — A morphological mutant of the self-fertilizing, hermaphroditic nematode species Rhabditis briggsae has been isolated – the first to be reported in the phylum Nematoda. It is designated micro and affects the gross appearance of the homozygous individuals by a general reduction in size, impairment of the egg-laying mechanism so that ova are laid so slowly as to accumulate and kill the hermaphroditic parent, and alteration in the structure of the male copulatory bursa. The mutation appeared among descendants of one of a series of isolated hermaphrodites kept at a temperature of 25° C for 18 hours during the time of sexual maturation. It is not yet possible to state whether or not a causal mechanism between heat and mutation may exist for these forms. The inheritance of the micro gene follows a single factor pattern and is recessive and not sex linked. Experiments have been done in which micro hermaphrodites have been successfully outcrossed with wild type males. The self-fertilized progeny of the micro parent can be readily distinguished from the cross-fertilized hybrid progeny by the mutant phenotype of the former vs. the wild phenotype of the latter.

O'MARA, JOSEPH G., U. S. Department of Agriculture and University of Missouri, Columbia, Missouri: The structure of chromosome I of Secale cereale. — In progenies secured by selfing Triticum vulgare (n = 21) to which a single chromosome from Secale cereale (n = 7) had been added, two kinds of Secale univalents were found. One was isobrachial, the other heterobrachial. The presumption was made that the heterobrachial chromosome was the normal chromosome, and that the isobrachial chromosome was an isochromosome of two long arms. This presumption did not accord with the phenotypes of the individuals in various progenies. (The phenotype determined by this chromosome is that known as "hairy neck"

or "behaarten Halmen.") The explanation of the inconsistencies lay in the fact that the obvious constriction of the chromosome is not the kinetochore constriction. The true kinetochore constriction is subterminal and comparatively obscure. Consequently, the chromosome considered to be an isochromosome is actually the normal chromosome of Secale cereale, and the apparently heterobrachial chromosome is telocentric for the long arm. This explanation has been verified by deriving various progenies in which the chromosome constitutions correspond to the predicted phenotypes. The Secale cereale chromosome has been isolated in the following forms: (1) the normal chromosome, which is markedly heterobrachial but seems isobrachial, (2) the telocentric chromosome which represents the long arm but appears heterobrachial, (3) the telocentric chromosome representing the short arm, (4) the isochromosome of the short chromosome arm in duplicate, and (5) the isochromosome of the two long arms – this chromosome has three constrictions, two secondary constrictions which are very obvious and the kinetochore constriction which is relatively obscure.

PATAU, KLAUS, University of Wisconsin, Madison, Wis.: A correlation between separation of the two chromosome groups in somatic reduction and their degree of homologous segregation. — The term "reductional grouping" (RG) is used for a nuclear condition showing otherwise normal mitotic chromosomes separated into two (or more) numerically equal or unequal groups. If sufficiently apart both groups may undergo anaphase separately, thus producing four nuclei with reduced chromosome sets. In equal RG's from squash preparations (root tips of Rhoeo and Allium) measurements were made: (1) of chromosome distances within and between groups, (2) of chromosome lengths. From the first a value, S, characterizing the degree of group separation, was computed; from the second another value, H, expressing the degree of above-random length agreement between the group totals, (H=1 if these are equal). In prophases H varied around the random expectation without relation to S. All RG's at meta- or anaphase, and most of those classed as "prometa- or metaphase," showed a highly significant strong positive correlation between S and H, the latter sometimes approaching 1. This correlation cannot be due to systematic errors. A close relation between separation and small differences of total lengths as such appears extremely unlikely. It is therefore concluded that the group separation is closely determined by what may be called, without physical implications, "homologous repulsion." It seems that RG's originate at prophase as random irregularities and that later, presumably during formation of the mitotic spindle(s), only those are kept apart which happen to have a sufficient degree of homologous segregation. Reduced nuclei derived from RG's may, therefore, be expected to have a more or less complete genome.

PAULEY, SCOTT S., Harvard University, Harvard Forest, Petersham, Mass.: Ecotypic variation in Populus species. — In recognition of the necessity of accumulating basic data on the degree of variability which exists in the wild populations of our native tree species as an initial step in tree breeding programs, clone collections representing various geographic origins of some important species in the genus Populus were undertaken by the Cabot Foundation for Botanical Research in the fall of 1947. These were increased in the fall of 1948 and now number more than 1,000. On the basis of preliminary observations made on this material while in the propagation beds it is apparent that there are marked variations between clones of different geographic origin which must be attributed to genetic differences. Many of the conspicuous variations are related to the timing of certain physiological functions, especially time of leaf emergence ("flushing") in the spring and time of growth termination and leaf fall in the autumn. P. trichocarpa clones, for instance, of Alaskan origin terminate growth and harden wood the latter part of August when grown at the latitude of Boston, whereas trichocarpa clones of southern Oregon origin (ca. the same lat. as Boston) remain in active growth until the middle of October. These characteristics are also reflected in the total growth per season since all clones of far northern origin propagated in this study are uniformly dwarfed when grown in the shorter day of the Boston latitude. Other apparent ecotypic variables include resistance to Melampsora rust, growth rate, branching habit, stem form, leaf and bark colors and leaf shape.

PLOUGH, H. H., Amherst College, Amherst, Mass.: Penicillin isolated biochemical mutations in Salmonella typhimurium: their proportionality to X-ray dosage, and their failure to show recombination. — For some months our group (consisting of Helen N. Young, Madelon Grimm, Myron Saltz, Martin L. Vogel and others) has concentrated on isolating biochemically deficient strains of Salmonella typhimurium from suspensions subjected to X-radiation and then screened with penicillin according to the method of Davis. The original strain was used by Gowen in his studies of the genetic basis of virulence, and was derived from a single cell isolation by Zelle. Successive suspensions are X-rayed at dosages from 11,000R to 57,000R units, incubated 24 hours in complete medium, centrifuged, washed, resuspended in minimal medium (salts plus glucose) containing 200 units of penicillin per ml. and again incubated for 48 hours. This treatment kills most of the parent type organisms and from dilution plates made on complete medium 100 colonies at random are isolated. Preliminary platings showed that the X-radiation killed from 60% at 11,000R to more than 99% at 57,000R. Tests by the paper disc method of assay of the clones isolated as noted gave 9% biochemical mutants from the unradiated controls increasing up to 80% of mutations from the suspensions receiving the maximum

X-radiation dosage. In all we have over 250 mutant strains each of which requires one or more specific nutrilites for growth which the parent strain does not need. Specific amino acid requirements are most numerous, with purine requirements second. The most frequent amino acid requirements are cysteine, histidine, methionine, proline, tyrosine, leucine, threonine and valine. Several tests for possible sexual fusion and recombination by the method of Lederberg have proved entirely negative, perhaps indicating that Salmonella is a haploid uninucleate cell. Many interesting biochemical interactions are being further investigated.

POMPER, SEYMOUR, Oak Ridge National Laboratory, Oak Ridge, Tenn.: Genetic studies on yeast. — A strain of Saccharomyces cerevisiae Hansen has been investigated genetically. Haploid strains of opposite mating type (Lindegren's a and alpha) were exposed to ultraviolet irradiation, and mutants were recovered which required various metabolites. These include adenine, uracil, histidine, tryptophan, methionine, thiamine, and pantothenic acid. Techniques involving the recovery of prototrophic diploids were employed (a) to recover heterozygous diploids from crosses of marked strains and (b) to distinguish between phenotypically similar, but genotypically different, mutant alleles. Two nonallelic uracilless, five nonallelic adenineless (three of one type), and at least three nonallelic methionineless mutants have been tentatively established. Preliminary investigations have been carried out to determine the nature of the biochemical blocks in the nonallelic series. By microdissection, it was possible to recover the four products of meiosis, and to follow the segregations of mutant genes in various crosses. The results thus far obtained indicate that these characters segregate in regular Mendelian fashion, as though controlled by single genes.

POULSON, D. F., Yale University, New Haven, Conn.: Chemical differentiation of the larval mid gut of Drosophila. — The accumulation of mineral elements such as iron and copper in certain cells of the larval mid gut of flies can be readily demonstrated by histochemical methods. Forty species representing 5 of the 7 subgenera of the genus Drosophila and 12 of the species groups have been studied for iron using the Prussian blue reaction with potassium ferrocyanide and for copper using the reaction with sodium diethyldithiocarbamate. Characteristic differences in the pattern of iron accumulation exist within the subgenera between species and, in some cases, between strains. The gut regions concerned are: the anterior mid gut exclusive of the caeca; the posterior region of the middle mid gut; and a large section of the posterior mid gut. The extent and pattern of accumulation is quite constant for a given strain on a given concentration of inorganic iron in the medium. It is clear from experiments with D. melanogaster and D. virilis that the amount of stored iron is proportional to the iron content of the medium over a considerable range. There are indications that the stored iron is in the form of ferritin

or a similar complex. The genetic aspects of these differences are being studied. Copper accumulation is limited to certain cells of the middle mid gut in larvae (of all species studied) raised on copper supplemented media. The mechanism clearly differs from that for iron, and in freshly dissected guts the copper-accumulating cells give a characteristic orange-red fluorescence in ultra-violet light which is dependent on the copper concentration in the medium. A similar fluorescence is given by the livers of a number of crustacea and cephalopods from which a fluorescent heat stable copper-containing material is now being isolated for further study.

POWERS, E. L. and CARLENE RAPER, Argonne National Laboratory, Chicago, Ill.: Responses induced by a nitrogen mustard compound in Paramecium aurelia. — Death of cells prior to autogamy, suppression of fission, and death after autogamy were measured in Stock 51 of Paramecium aurelia as functions of increasing time of exposure to a solution of methyl-bis (B-chloroethyl) amine hydrochloride (HN2). Two-day exautogamous clones were held 18 hours in a salt solution containing $CaCl_2$ and $MgCl_2$ (.002% each) and .008 molar $KH_2PO_4$ with NaOH to pH 8 previous to exposure in a similar solution with .002 molar HN2. Times of exposure were hourly, from 1 to 6 hours. Death of cells, usually occurring with 48 hours, was related to time of exposure in typical sigmoid fashion, 50% dying after 5 hours exposure. The time after exposure to first division of surviving cells increased rapidly with increasing duration of exposure up to 4 hours (60 hours delay), the effect not increasing significantly thereafter. In each of the 8 experiments to date, death after induction of autogamy in surviving cells rose with time of exposure to HN2 to a maximum and then decreased, the mean being a maximum of 71% after 5 hours exposure, followed by a decrease to 59% after 6 hours.

POWERS, E. L., and DEBORAH SHEFNER, Argonne National Laboratory, Chicago, Ill.: Effects of high dosages of X-rays in Paramecium aurelia. — Stock 51 Paramecium aurelia was given in culture solution dosages of unfiltered 50 kv X-rays up to 1,000,000 r at 62,200 r per minute. Fifty per cent of the cells died after 650,000 r, and fission rate of survivors was 50% of control rate at the same level. Death after autogamy increased to 98% at 50,000 r, followed by a decrease to about 80% at 250,000 r and a subsequent slow rise at higher doses. These responses are like those observed following mustard treatment. However, in contrast, the peak in the autogamy curve induced by X-rays is higher, and it occurs at doses below those causing physiological effects of any magnitude.

RILEY, HERBERT PARKES, and NORMAN H. GILES, Jr., Oak Ridge National Laboratory, Oak Ridge, Tenn.: The effect of oxygen on the frequency of x-ray induced chromosomal aberrations in Tradescantia microspores. — A study has been made of the effect of oxygen on the

frequency of X-ray induced chromosomal aberations (interchanges and interstitial deletions) observed at the four-to-five-day interval following irradiation of microspores. Initial comparative exposures made in air, in nitrogen, and in oxygen demostrated that aberration frequencies were markedly reduced in nitrogen and increased in oxygen as compared with air. Further test exposures in a vacuum and in other gases such as helium and argon also resulted in greatly reduced aberration frequencies, indicating that the availability of oxygen has a marked effect in increasing the radiosensitivity of Tradescantia chromosomes. Additional experiments have been performed comparing the aberration frequencies in two sets of inflorescences – one kept in a vacuum both during and following irradiation and the other irradiated in a vacuum with pure oxygen being introduced under pressure immediately following the radiation exposure. The failure to find any increased aberration frequency in the second set, to which oxygen was added after irradiation, suggests that the effect of oxygen in increasing aberration frequency is exerted on the initial breakage mechanism rather than on the recovery process.

ROMAN, HERSCHEL, University of Washington, Seattle, Wash.: Factors affecting mitotic nondisjunction in maize. — The B-type chromosome in maize frequently undergoes nondisjunction in the second division of the miscrospore. Several translocations with members of the basic complement have been obtained, involving breakages at various points in the B chromosome. In each case only the translocation chromosome which bears the B centromere exhibits nondisjunctional behavior. For this reason the centromere has been regarded as the sole chromosomal element responsible for nondisjunction. Recent evidence, however, suggests that a part or perhaps all of the main heterochromatic segment, which comprises the distal two-thirds or so of the chromosome in pachytene, may be a second chromosomal factor affecting nondisjunction. In TB-4a, one of the translocation chromosomes ($4^B$) carries the centromere of chromosome 4 and the entire heterochromatic segment of the B chromosome. The other translocation chromosome ($B^4$) contains the B centromere and the distal three-fourths of the short arm of chromosome 4. In the second division of microspores which contain the $4^B$ and $B^4$ chromosomes, the frequency of nondisjunction of the $B^4$ chromosome may be as high as 80%. In microspores which do not have the $4^B$ chromosome but have rather a normal chromosome 4 and the $B^4$ chromosome, the latter undergoes nondisjunction rarely if at all. The 4 $B^4$ microspore differs from the $4^B$ $B^4$ microspore in two respects: (1) it lacks the heterochromatic segment of the $4^B$ chromosome and (2) it contains a duplication of the segment of chromosome 4 found in the $B^4$ chromosome. Tests now underway are designed to determine whether the failure of nondisjunction in the 4 $B^4$ microspore is due to the absence of the heterochromatic segment or to the presence of the duplication.

RUBIN, B. A., Brookhaven National Laboratory, Upton, L. I., N. Y.: The pattern and significance of delayed phenotypic expression of mutations induced in E. coli by absorbed $P^{32}$. — Biologically absorbed radioactive phosphorus ($P^{32}$) has been shown to induce significant increases in the proportion of streptomycin resistant cells among growing populations of Escherichia coli (strains B and B/r). Great differences in the absorption of $P^{32}$ by the bacteria have been produced by varying carrier phosphorus. Calculations show that large increases in concentration of $P^{32}$, in a cell of bacterial dimensions, produce insignificant changes in specific ionization. The increase in mutation rate, however, is disproportionately high. When bacteria were grown in solutions of $P^{32}$ (up to 8 mc per ml) the mutation rate increased with dose. But in all cases the relative size of the mutant population changed upon further cultivation. The pattern of change (diverse rates of increase, sometimes followed by decrease) varied with the concentration of $P^{32}$ and with the amount of absorption, as controlled by carrier phosphorus levels. The complete phenotypic expression of the mutations produced seemed also to depend upon the number of bacterial divisions, since similar changes did not occur in resting cultures exposed in the same way, for similar periods. These patterns indicated a quantitative, as well as qualitative difference in distribution of mutant types produced by an effect which can not be accounted for by ionization alone. Calculations indicate that this effect may be attributed to the transmutation of $P^{32}$ to $S^{32}$.

RUSSELL, W. L. and JOSEPHINE S. GOWER, Oak Ridge National Laboratory, Oak Ridge, Tenn.: Offspring from transplanted ovaries of fetal mice homozygous for a lethal gene (Sp) that kills before birth. — Although the evidence for recessive prenatal lethals is generally considered adequate, the clinching proof, a breeding test of the lethal type itself, has been lacking. The first test of this kind has now been made by transplanting embryonic ovaries, a method reported earlier, and using the splotch (Sp) gene in the mouse. Heterozygous, Sp sp, mice are white-spotted; presumed homozygous, Sp Sp, embryos die, with characteristic abnormalities, at about day 14 in gestation. Ovaries from the abnormal embryos at day 13 1/2 in gestation were transplanted to sp sp hosts which were then mated to sp sp males. The stocks developed for this test provided host-graft compatibility and contained genetic markers that would distinguish offspring of transplanted ovaries from offspring of regenerated host ovaries. All the offspring from the transplanted ovaries were found to be Sp sp, thus proving the embryos to be homozygous Sp Sp. The method should prove useful not only in tests of other lethals, both gene and chromosomal, but also in the genetic identification of all degrees of expression of a particular lethal. Thus, in the present investigation, an embryo exhibiting only a tail defect, in contrast to the usual extensive abnormality of the neural tube in Sp Sp homozygotes, was shown to belong to the homozygote class. The method

can, furthermore, aid in the analysis of the action of lethal genes. The homozygous splotch combination, for example, is here shown not to upset the development of a potentially fertile ovary.

SCHULTZ, JACK, Institute for Cancer Research, Fox Chase, Philadelphia, Pa.: Interrelations of factors affecting heterochromatin-induced variegation in Drosophila. — The variegation induced in loci transposed to heterochromatin is known to depend upon two factors: distance from the point of rearrangement, and the nature of the allel at the locus itself. In an experiment reported briefly some years ago, genetic modifiers of variegation were detected and analyzed. Among these modifiers some were found to be specific for the effect upon the locus (type of mottled allel manifested). Others affected both the range of the position effect and the type of allel shown in the mottling. The modifiers are located throughout the chromosomes, important ones being found in the Y and in other heterochromatic regions. The grade of variegation in any particular stock of a rearrangement is the resultant of the effects of the different modifiers upon it, a situation similar to that obtaining for other phenotypes. Examples of interaction will be given, and the analysis of the diverse effects of the modifiers shown. The heterochromatin induced variegation process is thus to be compared with other instances of genotypic control of the behavior of the chromosomes. The effects on the grade of variegation of the balance of nutritional factors in experiments using a chemically defined medium will be discussed; the multiplicity of factors already found corresponds to that expected from the genetically complex determination of variegation grade.

SHULL, A. FRANKLIN, University of Michigan, Ann Arbor, Mich.: Imitation of specific pattern characters of Hippodamia quinquesignata through residual variation in H. convergens. — The pattern of Hippodamia quinquesignata differs from that of H. convergens in having an anterior band and a postmedian band, each in place of two spots, and in sometimes lacking, between these bands, the lateral spot which is practically always present in H. convergens. A single hibernating mass from Pullman, Washington, contained variations out of which a fair imitation of quinquesignata could be constructed. An anterior band identical with that of quinquesignata was found in one male. It was dependent on one dominant gene. If this band was introduced to convergens by a species cross, that cross must have happened some generations earlier since all traces of quinquesignata genitalia were lacking in that male. A postmedian band not identical with that of quinquesignata was constructed from several genes which may contribute to the band in that species; but something, probably one gene (as determined from earlier work), was missing from this band. The lateral spot, formerly regarded as a separate character, is now found to be instead a part of the expression of the anterior band. Some individuals with this band show the spot, others lack it, depending probably on something

in the genetic background. This alteration of the status of the lateral spot reduces by one the number of genes differentiating the two species, which had been regarded as possibly as few as five. The best imitation obtained would be identified by specialists not examining genitalia as H. quinquesignata if taken in the appropriate area.

SPARROW, ARNOLD H., Brookhaven National Laboratory, Upton, L. I., N. Y.: Tolerance of Tradescantia to continuous exposures to Gamma radiation from Cobalt $^{60}$. — Plants of a clonal line of Tradescantia paludosa were exposed under field conditions to continuous gamma radiation (1.1 and 1.3 Mev) of approximately 122, 30.4, 7.5, 1.8, 0.41 and 0.084 r per day, for 8, 16, 32 and 64 days. Dosage rates of 122 and 30.4 r per day permitted little or no growth, while fairly normal growth occurred at 7.5 r per day or less. Complete pollen abortion was produced after 16 days at 122 r per day. Chromosome breaks as such were not scored, but micronuclei at the microspore resting stage were counted. These are assumed to represent acentric fragments which lagged and remained in the cytoplasm at second telophase. A significant increase in the frequency of micronuclei was obtained with as low as 84 mr per day after 32 days exposure (total dose 2.69 r). Dosage curves for various exposure times will be presented. An attempt will be made to relate growth-inhibiting and lethal doses to the percentage of microspores containing micronuclei.

SPARROW, A. H. and E. CHRISTENSEN, Brookhaven National Laboratory, Upton, L. I., N. Y.: Non-random distribution of chromosome fragmentation in unirradiated Trillium. — An unusual type of spontaneous fragmentation has been found in a plant of Trillium erectum. There is considerable variation from anther to anther though some appear normal. In the most extreme case about 4% of the microsporocytes show aberrations. However, within this small group of cells chromosome fragmentation reached an extreme of about 30 breaks per cell. This is equivalent to that produced by a dose of at least 100 r at a highly sensitive stage or of not less than 5,000 r at a stage of low sensitivity. Statistical analysis shows the distribution of break frequency in the abnormal cells to differ significantly from that expected in irradiated material. The reason for this difference is not understood. Other abnormalities such as "fused" microsporocytes indicate that a physiological disturbance may be responsible. The seriousness of this type of spontaneous fragmentation in radiation experiments involving the use of chromosome breaks as a measure of radiation effect is obvious.

SPARROW, A. H. and RHODA C. SPARROW, Brookhaven National Laboratory, Upton, L. I., N. Y.: The frequency of spontaneous chromosome breakage in Trillium. — The frequency of spontaneous chromosome breakage in Trillium erectum has been determined by counts made at

first meiotic anaphase and at microspore metaphase. These will be compared with the frequency of micronuclei observed at the tetrad and postmeiotic interphase stages. Statistical analysis will be presented to show the significance (if any) of differences in the frequencies observed at various stages within a plant and also the differences between plants, between buds within a plant and between anthers within a bud. The results of such an analysis will be exceedingly useful in determining significant changes in chromosomal aberrations induced experimentally by irradiation or by chemical mutagens.

STOUT, A. B., New York Botanical Garden, New York, N. Y.: The genetics of unilateral hybridizations in Petunia. — Members of Petunia integrifolia produce no capsules to pollen of P. parodii or P. axillaris; the reciprocals give viable seeds. P. parodii hybridizes with P. axillaris only when the former are the female members. The progenies of such unilateral hybridizations provide excellent material for studies of the genetics of the relative specificities that are involved. All PA hybrids were fertile with pollen of P. parodii and all AI hybrids were fertile with pollen of P. axillaris. Of back-cross progenies of AA ♀ x PA ♂, at least half of the members had unilateral sterility with pollen of P. parodii. Some members of back-cross progenies of AAI hybrids x 2n P. integrifolia had unilateral sterility with pollen of P. axillaris. All diploid PA hybrids were fertile with ♀ P. axillaris, but there was masked interspecific unilateral sterility for the segregated pollen that was P. parodii. The results of tests with AI hybrids as males were not uniform, presumably because of intraspecific incompatibilities. But the seven seedlings of AAI x AA parentage had unilateral sterility with pollen of P. integrifolia. It is concluded that the genetic constitution in pistils that effects unilateral sterility in hybridizations in Petunia is either not present or is dormant in any haploid portion of the diploid constitution that is transmitted to hybrids; but in the pollen tubes a haploid constitution is sufficient. Some resultants suggest cytoplasmic inheritance, but segregations and recombinations in back-cross progenies indicate genic heredity.

TAYLOR, J. HERBERT, University of Tennessee, Knoxville, Tenn.: Transition of meiosis to mitosis and visa versa in cultures of excised anthers. — Excised anthers of Podophyllum peltatum were floated on a sterile solution composed of 1 part raw coconut milk and 3 parts of 2 percent sucrose solution supplemented with vitamins. Anthers were fixed at intervals and examined in squash preparations or serial sections. In anthers excised at the preleptotene stage, a progressive decrease of the sporogenous tissue and an increase in parenchyma occurs. Tapetum fails to differentiate. Usually the sporogenous tissue near the center of each microsporangium maintains its identity until the microsporocytes divide. These are the cells in which a transition from a meiotic prophase to a

mitotic metaphase and anaphase may be observed. The early prophase stages fix similar to typical meiotic prophase, pachytene is typical except the chromosomes are not paired, the chromosomes are visibly single compared to the visibly double character of those of normal mitotic prophase, the prophase is very long compared to mitotic prophase, and the divisions are synchronized in clusters as in meiosis. In those cases in which meiosis had been initiated, pairing occurred and meiosis was essentially normal. Because of the progressive degeneration of the spore forming tissue, microspores were seldom formed. Tapetum differentiated and appeared to grow at the expense of sporogenous tissue in those anthers excised at the later stages. In cultures of Tradescantia anthers, excised before the initiation of meiosis, this modified division often occurs in the sporogenous tissue. Tapetal cells were also observed which had acquired features of meiosis. No pairing was present, but very clear major and minor coils were observed in metaphase chromosomes of these tapetal cells.

VICARI, E. M., Jackson Memorial Laboratory, Bar Harbor, Maine: Genetic aspects of the age threshold of susceptibility to sound-induced convulsions in mice. — A survey of behavior patterns and of age susceptibility to sound-induced convulsions and seizures was made on inbred strains of mice. First test-trials only were considered. Convulsions were induced by the ringing of an electric bell. The convulsive pattern and the age susceptibility varied with the strain. One pattern was of a violent nature, sudden attacks of frantic running followed by clonic and tonic convulsions and seizures ending usually in death. Another pattern was not as violent but followed the clonic-tonic type. This was seldom fatal. Still another pattern was of a static and paralytic nature and at times cataleptic like. This was never fatal. The age susceptibility threshold for the violent type was between 20-80 days with a peak at age 29-33 days showing a 92% individuals convulsing of which number 70% died. A second peak was at 45-49 days. The age threshold for the less violent type was 60-210 days with the highest per cent performance at 90 days. The paralytic pattern had an age threshold susceptibility around 90-180 days with peak susceptibility around 120 days. The characteristic behavior strain-types were described and an attempt made to record these by means of movie films.

WAGNER, R. P., C. H. HADDOX, R. FUERST, and W. S. STONE, University of Texas, Austin, Texas: The effect of irradiated medium, peroxide and cyanide on the mutation rate in Neurospora. — Biochemical mutations have been induced in Neurospora crassa at a rate significantly higher than the control rate by treating the conidia with nutrient broth which had previously irradiated with ultra-violet light, and also by treating the conidia with hydrogen peroxide and with potassium cyanide. The mutations were detected after having been passed through the sex cycle.

The rates induced by these various agents are not significantly different from one another, but are significantly lower than the rate induced by direct irradiation with ultra-violet. The types of biochemical mutants produced, as far as they have been identified, do not seem to be any different from those induced by direct irradiation. These results with Neurospora fully confirm in all important respects the results obtained by Stone et al (1947, 1948) and Wyss et al (1948) using bacteria, and definitely establish the production of mutants by indirect irradiation, as well as by hydrogen peroxide which has been postulated to be one of the mutagenic agents formed as a result of irradiation.

WARTERS, MARY, and A. B. GRIFFEN, Centenary College, Shreveport, La., and University of Missouri, Columbia, Mo.: The telomeres of Drosophila. — Terminal chromomeres have been identified in the salivary gland chromosomes of D. virilis and D. melanogaster. In virilis the telomeres of all chromosomes tend to synapse, forming a cluster of lightly-staining bodies, to which the distal ends of two or more chromosomes may remain attached after smearing. In melanogaster the telomeres likewise synapse, but may be resolved only with difficulty and do not maintain cluster form with tenacity. In both species the telomeres resemble the cemtromeres in their failure to reduplicate during salivary chromosome growth; accordingly, the number of telomeres per salivary chromosome is 8, or one for each end of each original sister strand of each homologue. No evidence is found for the presence of terminal heterochromatin in the cytological sense of the term, nor for the presence of any material other than the fundamentally homologous telomeres to account for terminal synapses between non-homologous chromosomes. The demonstration of telomeres as specialized cytological realities reenforces the doubt that Drosophila chromosomes are capable of survival after simple breakage.

WEIR, J. A., University of Saskatchewan, Saskatoon, Saskatchewan, Canada: Mechanisms of genetic resistance to infection in mice. — Six inbred mouse strains differing in resistance to mouse typhoid have been employed at the Iowa State College to determine the character basis for genetic resistance. Leucocyte count and blood pH have been shown to be positively correlated with resistance — J. Inf. Dis. 73: 40-56 (1943); 84: 252-274 (1949). Since present strains do not permit critical evaluation of independent contributions to resistance complex it is proposed that high and low leucocyte and blood pH lines be produced by selective breeding. Starting with MacArthurs random bred mice — Amer. Nat. 78: 142-157 (1944), with mean leucocyte count of 9559 (118 mice) and mean blood pH 7.45 (275 mice), $S_1$ progeny from selected parents have been produced as follows: 60 leucocyte high 9693, 65 leucocyte low 8672; 180 pH high 7.47, 165 pH low 7.42. Survivals among 40 random bred, 15 high pH and 16 low pH mice inoculated with S. typhimurium were 48%, 67% and 38% respectively. Survivals to date in a trial in progress are leucocyte low

26%, leucocyte high 33%, pH low 28%, pH high 44%. There was found to be no correlation between leucocyte count and blood pH in mice tested for both characters.

WHITE, MICHAEL J. D., University of Texas, Austin, Tex.: Cytological polymorphism in wild populations of western grasshoppers. — Three main types of cytological polymorphism are present in various species of the grasshopper genus Trimerotropis in western North America, either separately or in combination: (1) supernumerary chromosomes, (2) supernumerary chromosome regions, (3) centromere shifts. Supernumerary chromosomes are present in many species, but are rare in most populations (for example, in a certain population of T. latifasciata approximately 1 in 9 individuals carries a supernumerary chromosome). Supernumerary chromosome regions have been studied especially in a population of T. bilobata in which one of the smaller chromosomes exists in three lengths, the two longer types of chromosomes containing heterochromatic segments which are lacking in the shortest type. Centromere shifts have been studied in T. sparsa, an unusually complex species with at least 3 chromosomal races. On the alkali flats of Nevada and Oregon a form occurs which is cytologically monomorphic (apart from occasional supernumerary chromosomes), having 3 pairs of metacentric autosomes and 8 pairs of acrocentrics. Populations of the same species from Idaho, western Colorado and northern New Mexico are cytologically polymorphic, the number of acrocentric and metacentric autosomes varying from one individual to another. At two localities in northwestern Colorado a "race" occurs in which there are only 10 instead of 11 pairs of autosomes. Highly significant differences in the mean numbers of acrocentric and metacentric chromosomes exist between populations from Craig and Whitewater, Colorado and Pojoaque, N. M.

WHITING, ANNA R., University of Pennsylvania, Philadelphia, Penn.: The non-induction of mutations by x-rayed cytoplasm. — Unfertilized Habrobracon eggs develop into haploid gynogenetic males. Of several thousand irradiated in first meiotic metaphase, none have hatched after any dose higher than 2,500 r. Cytological observations demonstrate a consistent pattern of chromosome injury. When severe, this injury retards movement of egg pronucleus after which the egg, fertilized by an untreated sperm, may develop into a haploid androgenetic male. Eggs are fertilized between one and seven hours after exposure. From 6,714 x-rayed unmated wild type females crossed to untreated recessive males, 170 recessive androgenetic males have been obtained. These developed in egg cytoplasm exposed to doses ranging from 1,350 to 65,000 r, the latter dose about twenty-five times as great as that fatal to egg chromosomes in this stage. No mutations appeared in any of these males and of thirty-two tested, all were fully fertile and transmitted only paternal

traits. Of 2,389 half-sisters of androgenetic males developed from eggs x-rayed in first meiotic prophase and fertilized by untreated sperm, 129 or 5.39% produced mutant sons. This differs significantly from the results obtained on androgenetic males. Evidence supports the theory that x-rays induce visible mutations directly in the chromosomes.

WHITING, P. W., University of Pennsylvania, Philadelphia, Penn.: Mutant types in Mormoniella. — Wild type eye color of this chalcidoid wasp is reddish brown. X-ray mutants are oyster white, oy, lacking red pigment but retaining dark, and several reds including garnet, ga, vermilion, vm, scarlet-4 (female-sterile), st-4, and scarlet, st, listed with black fleck of decreasing size, and tomato, to, which is of more uniform color. oy crossed with the reds gives wild type (complementary) although vm, st-4 and st are allelic with it and therefore might be symbolized $oy^{vm}$, $oy^{st-4}$ and $oy^{st}$ respectively. oy masks both ga and to and ga to is light orange. ga, to and oy with its red alleles segregate independently. Wild type body color is iridescent bronze-green in males, darker in females. Several x-ray mutant types (sterile) range from greenish-blue through purple to blue-black, suggesting decreasing amounts of yellow pigment. A single fertile purple, pu, body-color, taken wild at Woods Hole, shows linkage with the oy series. $F_2$ males from st/oy compound females crossed with pu males were + 153, st 320, oy 811, pu 1045, st pu 47, oy pu 113, showing 12.6% crossovers. Structural mutants include shredded wings and short legs, both sterile. Several mosaics, both males and females, and two gynanders have been found. Microcephaly, ranging to near acephaly, occurs irregularly in both sexes with incidence differing widely in different stocks.

WHITTINGHILL, MAURICE, Oak Ridge National Laboratory, Oak Ridge, Tenn.: Some effects of gamma rays upon recombination frequencies and on crossing over in Drosophila melanogaster. — In testcrosses of heterozygous cu/ ru h th st + sr $e^s$ ca females irradiation by 4,000 r of gamma rays increased recombinations in the spindle attachment region to 2 1/3 times the control value. Other increases of successively smaller magnitudes relative to control values were found in the flanking regions grading down to terminal decreases. The coincidence curve for all treated testcrosses showed a similar regular shape when the absolute changes were divided by the possible increases. Coincidences were higher in all regions in treated material. These increases also graded up to a peak at the spindle region. Both these curves are qualitatively predictable from the hypothesis that irradiation induced a rather small amount of genetic crossing over in oogonial cells. There was a considerable variation between females in the amount of response and also in the place of response to irradiation, resembling previous results from treated males. 4,120-r gamma-ray treatment of S $L^4$ /Cy Pfd females produced "clusters" of crossovers, of appreciable size in some families. A similar treatment

of males produced crossovers also in a nonrandom manner appropriate to spermatogonial crossing over. The implications to the collection of data for standard maps are considered.

WHITTINGHILL, MAURICE, University of North Carolina, Chapel Hill, N. C.: The non-random distribution of spontaneous crossovers from Curly inversion heterozygotes of Drosophila melanogaster. — Eighteen Cy lt $L^4$ heterozygotes having 7,477 offspring in two-day coded cultures produced 28 spontaneous crossovers non-randomly. There were too many similar pairs and triplets. Considering only families missed and one family having four region-one crossovers, a joint probability of $1.6 \times 10^{-8}$ was computed. Recombinations decreased greatly with age indicating that the age effect is created in oögonial cells rather than during meiosis.

WITKIN, EVELYN M., Carnegie Institution of Washington, Cold Spring Harbor, N. Y.: Effect of nucleic acid on phenomic lag in Escherichia coli. — When a series of 50 tubes of broth containing 0.5% sodium ribonucleate (Schwartz) is inoculated with strain B/r of Escherichia coli, the cultures obtained after 48 hours of incubation contain, on the average, a significantly higher number of mutants resistant to bacteriophage T1 than a similar series of cultures grown without nucleate. The higher number of mutants is not due to an increased rate of mutation during growth in nucleate. Nucleate cultures and broth controls do not differ in frequency of phage-resistant mutants at the end of the logarithmic growth phase. The difference appears only as the cultures are incubated in the "stationary" phase. The effect is not due to differential stimulation of the division of phage-resistant mutants, nor to more active total division of the nucleate cultures. Analysis of the data suggests that nucleate brings out the phenotypic expression of spontaneous delayed mutations, which would ordinarily be detected as resistant only after passing through one or more divisions. This hypothesis is supported by experiments using ultraviolet-irradiated populations known to contain large numbers of induced delayed mutations. Under certain conditions, nucleate can take the place of division in bringing out the phenotypic expression of ultraviolet-induced delayed mutations. Similar results have been obtained with high concentrations of casein hydrolysate.

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